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Using the elementary operations of sequential population analysis to display problems in catch or survey data

Geoffrey T. Evans

Abstract: A sequential population analysis is built from two elementary operations: accounting for deaths in a cohort during a year, and comparing abundance indices of two cohorts at the same age. Inferring the population of one cohort at age a from another cohort at age $a + 1$ uses both operations, and it should not matter which one is performed first. In analyses of four groundfish stocks from Atlantic Canada, accounting first for deaths gives systematically higher estimates of present numbers. The discrepancies will produce a retrospective pattern. It is not possible to identify a precise cause, or remedy it, but the problem probably involves a change over time in some quantity that has been assumed constant.

Résumé : On construit une analyse séquentielle de la population à partir de deux opérations élémentaires : le compte des mortalités dans une cohorte au cours d'une année et la comparaison des indices d'abondance de deux cohortes du même âge. Ces deux opérations sont nécessaires pour déterminer la population d'une cohorte d'âge a à partir d'une autre d'âge $a + 1$, et l'ordre dans lequel ces deux opérations sont effectuées devrait être indifférent. Lors d'analyses de stocks de poissons de fond du Canada atlantique, le fait de compter les mortalités d'abord a donné des évaluations systématiquement supérieures par rapport aux valeurs réelles, et ces écarts produiront un profil rétrospectif. Il n'est pas possible de trouver une explication précise ou une solution à ce problème, qui est probablement dû à la variation en fonction du temps d'un certain facteur supposé constant par hypothèse.
[Traduit par la Rédaction]

Introduction

Sequential population analysis (SPA) is a method for working out how many animals there were, and are, if we know how many died at each age. From early ideas of Fry (1949) and Gulland (1965) it has been developed into a standard method for assessing many fish stocks. Mohn and Cook (1993) give a detailed account of current practice. Recently a problem has been noticed. As time goes on and more information becomes available, one would expect estimates of past numbers to change somewhat. What was not expected, though, is that these changes have been reductions in estimated numbers far more often than chance would suggest (Sinclair et al. 1991). This so-called retrospective pattern or problem has cast doubt on the method. There have been attempts to explain it in terms of the statistical "tuning" methods used to combine all the information into a single best estimate (Anonymous 1991; Myers and Cadigan 1995). In this paper I shall explore another approach, suggesting that the problem already exists in inconsistencies between the data and the models used to describe them, before any statistical combination is attempted. I present the elementary operations that underlie SPA, show how these operations suggest a simple consistency check for the data, and show how data from four groundfish stocks from Atlantic Canada fail this check.

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Elementary operations of SPA

SPA keeps track of the numbers $N(y, a)$ in a cohort born in year y that were alive at the start of the year in which they attained age a, by making repeated use of the following trivial fact:

$$(1) \quad N(y, a) = N(y, a + 1) + D(y, a).$$

where $D(y, a)$ is the number in the cohort that died at age a (Table 1). There is an age A (much older than the oldest age for which assessments are typically done) by which we can safely assume that there are no fish left in the cohort. I shall refer to cohorts that have reached age A as finished cohorts; it is convenient to index their year of birth by f rather than the general y. Thus $N(f, A) = 0$ and the numbers at successively younger ages are given by

$$(2) \quad N(f, a) = \sum_{k=A-1}^{a} D(f, k).$$

Cohorts that have not yet reached age A (call them active cohorts and index their birth year by h; they still have some harvestable population left) need an extra step: if $u(y, a)$ is an index proportional to the abundance, then

$$(3) \quad n(h, a) = N(f, a)u(h, a)/u(f, a) = N(f, a)r_{hf}(a)$$

is an estimate of the population $N(h, a)$ of an active cohort h at age a, which uses the finished cohort f at the same age for calibration. Knowing the deaths $D(h, a)$ in the active cohort, one can then infer its population at all other ages. For example, we can infer the recruitment $N(h, B)$ where B is the age at

Table 1. Notation.

Symbol	Description
a	Age of a cohort
A	Age at which the population of any cohort can safely be assumed to be 0
B	Age at recruitment, at which fish first become available for harvest
$D(y, a)$	Number of deaths in the cohort born in year y during the year in which it attained age a
$d(y, a)$	Estimate of $D(y, a)$
f	Year of birth of a finished cohort
h	Year of birth of an active (harvestable) cohort
$N(y, a)$	Number in the cohort born in year y alive at the beginning of the year in which it attains age a
$n(h, a)$	Estimate of $N(h, a)$ for an active cohort
$n_R(h, a), n_L(h, a)$	Estimates of $N(h, a)$ from $N(f, a + 1)$ using right- and left-turn inferences, respectively
$R_{hf}(a)$	Ratio of the true abundances $N(h, a)/N(f, a)$
$r_{hf}(a)$	Ratio of the abundance indices $u(h, a)/u(f, a)$
$u(y, a)$	Index of abundance proportional to $N(y, a)$. The constant of proportionality can depend on a but should not depend on y.
y	Year of birth of a general cohort

which the animals first become available for harvest, or at which they can first be reliably sampled:

$$(4) \qquad n(h, B) = \left(\sum_{k=A-1}^{a} D(f, k) \right) r_{hf}(a) + \sum_{k=a-1}^{B} D(h, k).$$

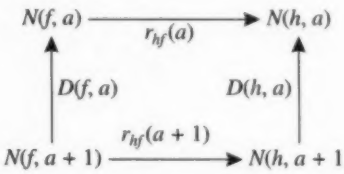
The genius of the method is that it provides a way to use abundance indices measured in previous years as additional, independent indices of this year's population. Every combination of age a and finished cohort f for which the calibration can be done gives a separate estimate of the recruitment, and the present numbers, of the active cohort h. These estimates can be combined statistically into a single best estimate, but it is also useful to examine the whole collection of estimates.

Consistency check

For example, we can check for internal consistency: there should be no systematic pattern depending on age or cohort of calibration. Now, recruitments estimated for the cod stock in the southern Gulf of St. Lawrence (NAFO divisions 4TVn) are larger when the calibration is made at younger ages. Figure 1 shows the estimate of the 1982 cohort; the pattern is similar for all other cohorts. (Commercial catch-at-age data and research vessel survey indices were taken from Hanson et al. (1992). Natural deaths were computed with the approximation of Pope (1972). The corresponding Fig. 1 in Evans (1994) was in error.) Clearly the underlying population or survey data (using the term data loosely to include the model of natural mortality) must be wrong in some way.

Let us examine the phenomenon more closely, and consider the simplest instance in which estimates can disagree: inferring the numbers in an active cohort at age a from the numbers in a finished cohort at age $a + 1$. Two steps are involved: calibrating indices between cohorts, and replacing deaths within a cohort. It should make no difference which of these steps is performed first. That is, we expect

$$\frac{n_R(h, a)}{n_L(h, a)} = \frac{N(f, a + 1) + D(f, a) r_{hf}(a)}{N(f, a + 1) r_{hf}(a + 1) + D(h, a)} = 1$$



where n_R refers to the estimate made by replacing deaths first (a right turn in the diagram) and n_L refers to the estimate made by calibrating first (a left turn). Figure 2 shows all ratios n_R/n_L that can be calculated for 4TVn cod, as a cumulative probability distribution. It combines information from all active cohorts on the same picture; each raw observation is used in many calculations so that the ratios are not statistically or even algebraically independent. Corresponding left- and right-turn estimates of the same quantity differ by more than a factor of 2 about a third of the time. Thus we can expect a large uncertainty in combined estimates. More worrisome, there is a large asymmetry in the graph: right-turn estimates are larger than left-turn estimates about two thirds of the time, and the median value of their ratio is 1.29.

How would this pattern affect a conventional sequential population analysis? Inferences from older calibration ages become possible only after the cohort has attained those ages. Thus, as time goes on, the smaller estimates of recruitment that come from calibrating at older ages will become available, and the single best estimate of recruitment will become smaller. This retrospective pattern is well-known for this stock (Hanson et al. 1992) and for many other stocks in the North Atlantic (Sinclair et al. 1991). Although it was first noticed in the results obtained with integrated methods of combining data statistically into a single estimate (so that in principle the pattern might have arisen from the method of combination), it is clear from Figs. 1 and 2 that the problem exists already in the data. Notice also that there is no reason to suppose that the later, lower estimates are any more correct than the early ones, only that both cannot be correct.

Possible causes

What might cause such an asymmetry? First, we know that something in the data must be changing with time; otherwise, any set of D's and u's could occur equally well for a finished or an active cohort, and so errors would be equally likely in each direction. As a simple example, suppose the same set of numbers occurred for both a finished and an active cohort; then it would make no difference which age a was used in eq. 4: the answers would be exactly the same. We might well get the wrong answer, but it would be the same wrong answer for any a and the right/left ratios would all be 1.

It is now time to acknowledge that, although standard assessment practice treats deaths $D(y,a)$ as known, in fact we only have estimates $d(y, a)$ that are subject to sampling error,

Fig. 1. Estimates of the recruitment of the 1982 cohort of 4TVn cod, as a function of the age at which the population index was calibrated.

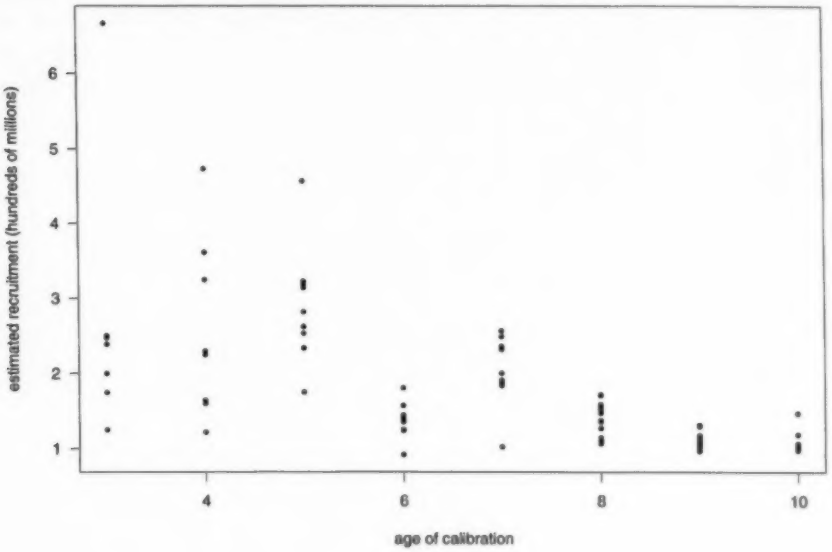
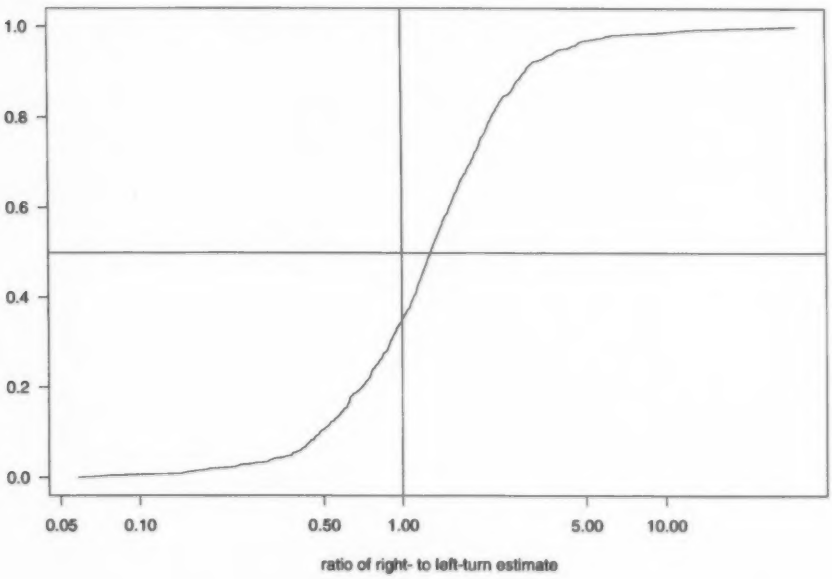


Fig. 2. The cumulative probability distribution of the ratio of right-turn to left-turn inferences for 4TVn cod.



incomplete reporting, inaccurate models of unreported deaths, etc. The question then is what could cause

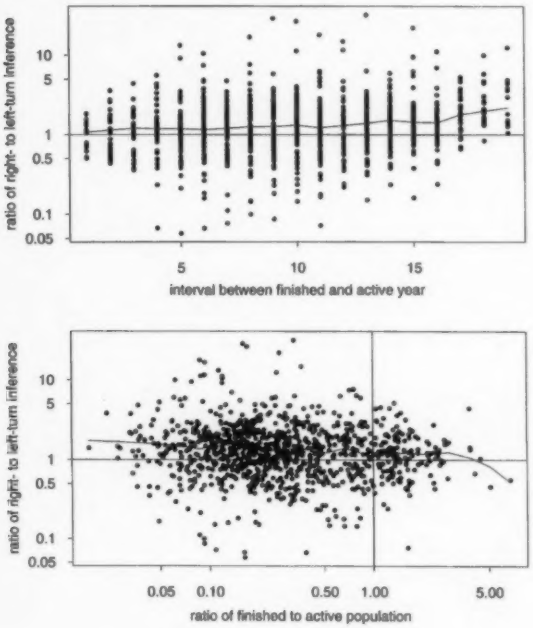
(5) $$n(f, a+1)r_{hf}(a+1) + d(h, a) > (n(f, a+1) + d(f, a))r_{hf}(a).$$

If certain of the observed values are exact then the inequality simplifies. For example, if we start with the true numbers $N(f, a+1)$ and the ratios of abundance indices $r_{hf}(a)$ are equal to the true population ratios $R_{hf}(a)$, so that only the deaths can be in error, then there is right-turn excess if

(6) $$(d(f, a) - D(f, a))\, R_{hf}(a) > d(h, a) - D(h, a).$$

Too high an estimate of deaths in a finished cohort, or too low in an active cohort (for example, an increase in the amount of

Fig. 3. The ratio of right-turn to left-turn inferences for 4TVn cod, plotted against (A) the number of years between the finished and active cohorts used in the inference and (B) the ratio of the population indices of finished and active cohorts used in the inference. A nonparametric regression line (lowess) gives an informal indication of trend. The population variable in B uses the geometric mean of the population indices at ages a and $a + 1$. Most ratios of finished to active population are less than 1, which means that a large stock is being calibrated from a small one.



underreporting of catch or in natural mortality as time goes on), leads to a right-turn excess. Another possibility: if everything except the ratios r is accurate then there is a right-turn excess if

$$(7) \quad (N(f, a+1) + D(f, a))(r_{hf}(a) - R_{hf}(a)) > N(f, a+1)(r_{hf}(a+1) - R_{hf}(a+1)).$$

Such a pattern could be produced if survey catchability increased in such a way that $r_{hf} > R_{hf}$ by an age-independent fraction (because $D > 0$). The change in either case might be smoothly increasing with time, or there might be a sharp change about the time of the transition from finished to active cohort. If the change is smooth, then there will be a gradual decrease in the right-turn excess as the time between finished and active cohorts decreases, tending to zero in the limiting case of no difference in years. Such a pattern can be seen for 4TVn cod (Fig. 3A).

Changes in error patterns over time, especially gradual, systematic changes, are unsatisfying. They can be "eliminated" by assuming some sort of a trend, but there is no way to know if the assumptions are correct: there will be many ways to mask the symptoms without addressing the malady. Moreover, there is no way to know if the trend will continue and therefore what to do about the most recent information. There is in principle another possibility that might turn out to be easier to handle: stocks are generally declining, and perhaps some unchanging source of error tends to produce a right-turn excess when calibrating a small stock from a large one. Such explanations are not intrinsically more likely to be true, but if they are true then there is more hope of doing something about them to get correct estimates. But for 4TVn cod there is a right-turn excess whether the finished cohort is larger or smaller than the active cohort, and the excess is most pronounced when the finished cohort is the smaller (Fig. 3B).

Other stocks

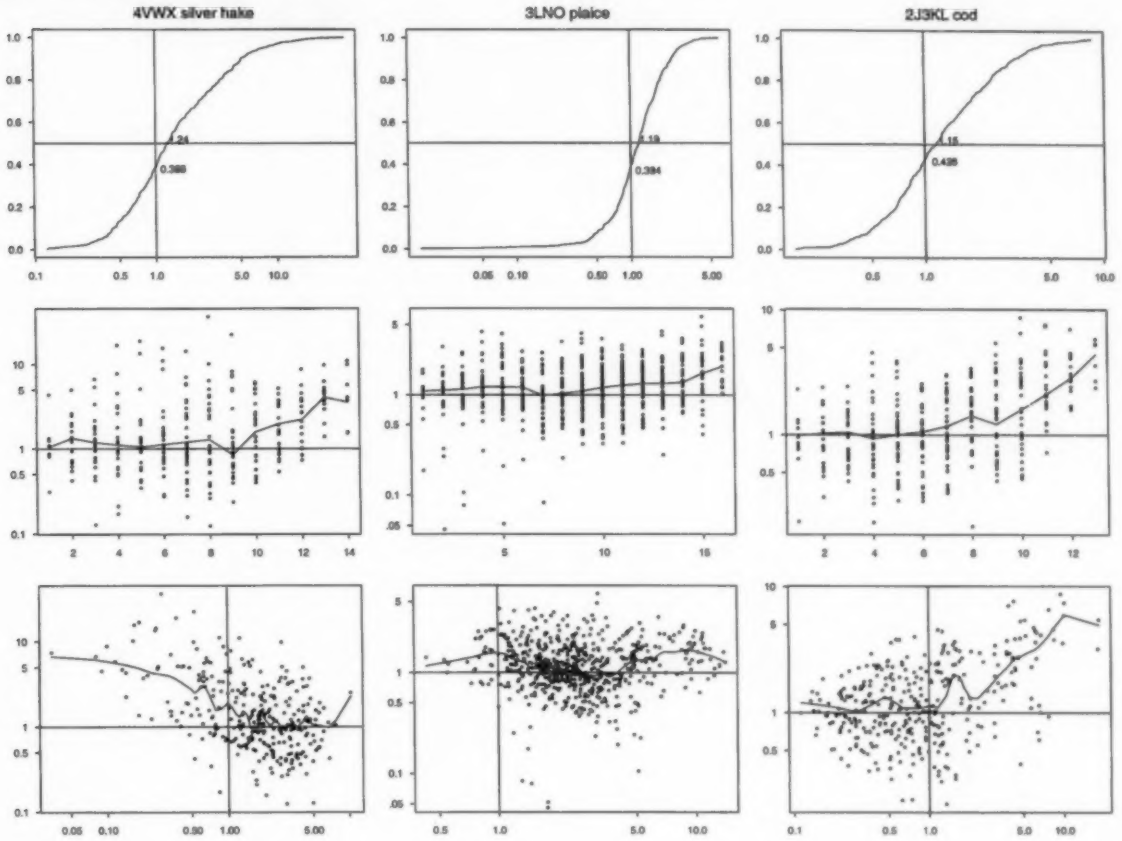
Figure 4 presents the distribution of excesses, and plots against time difference and population ratio, for three other stocks known to exhibit retrospective patterns: silver hake on the Scotian shelf (4VWX) (M. Showell, Department of Fisheries and Oceans, Bedford Institute of Oceanography, personal communication), plaice on the Grand Bank (3LNO) (W. Brodie, Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, personal communication), and northern cod (2J3KL) (D. Stansbury, Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, personal communication). For all of them there is a pronounced right-turn excess, and for all of them the excess increases with the difference in time between finished and active cohort. This supports the conclusion that some trend with time is involved. There is no systematic pattern with respect to difference in populations. Indeed, one might think of using these graphs to further classify retrospective patterns, according to whether the pattern was more pronounced when calibrating large stocks from small ones (the trend line has a generally negative slope, e.g., 4VWX silver hake) or the reverse (2J3KL cod). However, the lines produced by lowess smoothing, although eye-catching, have no measures of significance attached and may exaggerate random accidents.

Discussion

This work cannot firmly identify causes of retrospective patterns. The answers must lie in the details of how deaths are calculated and how surveys are conducted, details that do not appear in catch and survey tables. The purpose of the present work is to provide simple ways of demonstrating, at the most basic level, that the data on which SPA calculations are performed are inconsistent for some stocks, and to say something about the pattern of inconsistency. It can thus provide researchers into particular stocks with a better idea of where answers might be sought.

I am reconstructing numbers of fish from numbers of deaths. I thereby avoid any need to express fishing deaths as a fractional rate of change of the population ("fishing mortality"). There is also no need for simplifying assumptions about how fishing pressure is distributed over the year. In the examples, I made the assumptions of cohort analysis (Pope 1972) that are usual in assessing the stocks, but in practice it is just as easy to incorporate the pattern of fishing deaths directly, in as much temporal detail as known.

Fig. 4. The ratio of right-turn to left-turn inferences for other stocks. The first row corresponds to Fig. 2, the last two rows to Figs. 3A and 3B, respectively.



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References

Anonymous. 1991. Report of the working group on methods of fish stock assessments. C.M. 1991/Assess:25. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Evans, G.T. 1994. Disentangling the inferences of sequential population analysis can reveal underlying problems. C.M. 1994/D:7. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Fry, F.E.J. 1949. Statistics of a lake trout fishery. Biometrics, **5**: 27–67.

Gulland, J.A. 1965. Estimation of mortality rates. Annex to Arctic Fisheries Working Group report. C.M. Gadoid Fish Comm. 3. International Council for the Exploration of the Sea, Charlottenlund, Denmark.

Hanson, J.M., Chouinard, G.A., Nielsen, G.A., and Currie, L.G. 1992. Status of the 4T-Vn (Jan–Apr) cod stock in 1992. CAFSAC Res. Doc. No. 92/55. Canadian Atlantic Fisheries Scientific Advisory Committee, Dartmouth, N.S.

Mohn, R.K., and Cook, R. 1993. Introduction to sequential population analysis. Northwest Atl. Fish. Organ. Sci. Counc. Stud. No. 17.

Myers, R.A., and Cadigan, N.G. 1995. Statistical analysis of catch-at-age data with correlated errors. Can. J. Fish. Aquat. Sci. **52**: 1265–1273.

Pope, J.G. 1972. An investigation of the accuracy of virtual population analysis using cohort analysis. Int. Comm. Northwest Atl. Fish. Res. Bull. **9**: 65–74.

Sinclair, A.D., Gascon, D., O'Boyle, R., Rivard, D., and Gavaris, S. 1991. Consistency of some northwest Atlantic groundfish stock assessments. Northwest Atl. Fish. Organ. Sci. Counc. Stud. No. 16. pp. 59–77.

Comparison of mercury concentrations in liver, muscle, whole bodies, and composites of fish from the Red River of the North

Robert M. Goldstein, Mark E. Brigham, and Joseph C. Stauffer

Abstract: Carp (*Cyprinus carpio*) from four sites and channel catfish (*Ictalurus punctatus*) from one site in the Red River of the North in 1994 were analyzed for total mercury content. In carp, mercury concentrations differed among liver, muscle, and whole bodies (0.11, 0.31, and 0.18 μg/g wet weight, respectively), between large and small size groups, but not location. Mercury distribution in channel catfish tissues differed from that in carp. Liver and muscle tissue had similar mean concentrations; each was higher than whole-body concentrations (0.16, 0.18, and 0.11 μg/g, respectively). Mercury concentrations were not significantly different between the two size groups of channel catfish. Weighted-mean mercury concentrations from seven individual fish agreed closely (usually within 10%) with concentrations determined on physical composites of the same fish. The ratio of mercury in whole bodies to mercury in muscle was similar for both carp and channel catfish. Historical data indicate that this ratio may be applicable to other species and locations. The ratio of mercury in livers to whole bodies and muscle differed between carp and channel catfish, which may reflect physiological differences between different trophic groups.

Résumé : On a dosé le mercure total chez des carpes (*Cyprinus carpio*) de quatre sites de la rivière Rouge du Nord en 1994, ainsi que chez des barbues de rivière (*Ictalurus punctatus*) d'un site de la même rivière. Chez la carpe, on observait des variations des concentrations de mercure du foie, des muscles et de tout l'organisme (0,11, 0,31 et 0,18 μg/g de poids frais, respectivement) entre les groupes de grande et de petite taille, mais non en fonction de l'emplacement des sites. La répartition du mercure dans les tissus de la barbue différait de celle observée chez la carpe. On observait des concentrations moyennes semblables dans le foie et les tissus musculaires, qui étaient supérieures dans les deux cas à celles mesurées dans tout l'organisme (0,16, 0,18 et 0,11 μg/g, respectivement). Les concentrations de mercure observées chez les deux groupes de barbues de tailles différentes ne présentaient pas de différences significatives, et les concentrations moyennes pondérées de mercure de sept spécimens de poissons présentaient un bon accord (écart de moins de 10%) avec celles obtenues à l'aide d'échantillons physiques composites du même poisson. Les rapports entre le mercure de tout l'organisme et celui des muscles étaient semblables chez la carpe et la barbue. Les données historiques indiquent que ce rapport pourrait s'appliquer à d'autres espèces et à d'autres lieux. Le rapport entre le mercure du foie et celui de tout l'organisme (et celui des muscles) n'était pas le même pour la carpe que pour la barbue, ce qui pourrait refléter des différences physiologiques entre différents groupes trophiques.
[Traduit par la Rédaction]

Introduction

Previous studies of trace-element concentrations in fish tissue in the United States have relied on using composite samples for analysis (Schmitt and Brumbaugh 1990; U.S. Environmental Protection Agency 1992). Composite samples are used to maximize statistical power for estimating mean concentrations of contaminants (Tetra Tech Inc. 1987; U.S. Environmental Protection Agency 1989) while minimizing analysis costs.

Depending on the objectives of the study, various tissues from individual fish or whole bodies have been combined into a composite and analyzed. When the objective of the research is to determine the potential effect of consumption of fish on human health, the tissue analyzed is usually muscle (with the skin either included or removed); when the objective of the research is to determine effects of a contaminant on individual fish health, organs such as kidney, gill, or liver may be analyzed; and when the objective is to examine trophic interactions or bioconcentration and bioaccumulation, whole bodies may be analyzed. The choice of tissue for analysis also depends on the nature of the trace element under study. Some elements, such as cadmium, copper, lead, and zinc, concentrate in the liver and kidneys (Weatherly et al. 1980). Mercury in the methylated form, which accounts for approximately 95–99% of the mercury in fish tissue (Huckabee et al. 1979; Bloom 1992), is lipid soluble and concentrates more readily in some tissues than others (Eisler 1987).

Mercury is accumulated in fish primarily through ingestion (Phillips et al. 1980; MacCrimmon et al. 1983; Cope et al. 1990), which may account for 90% or more of the methylmercury accumulated (Spry and Wiener 1991). The distribution and final disposition of mercury in fish is not constant or

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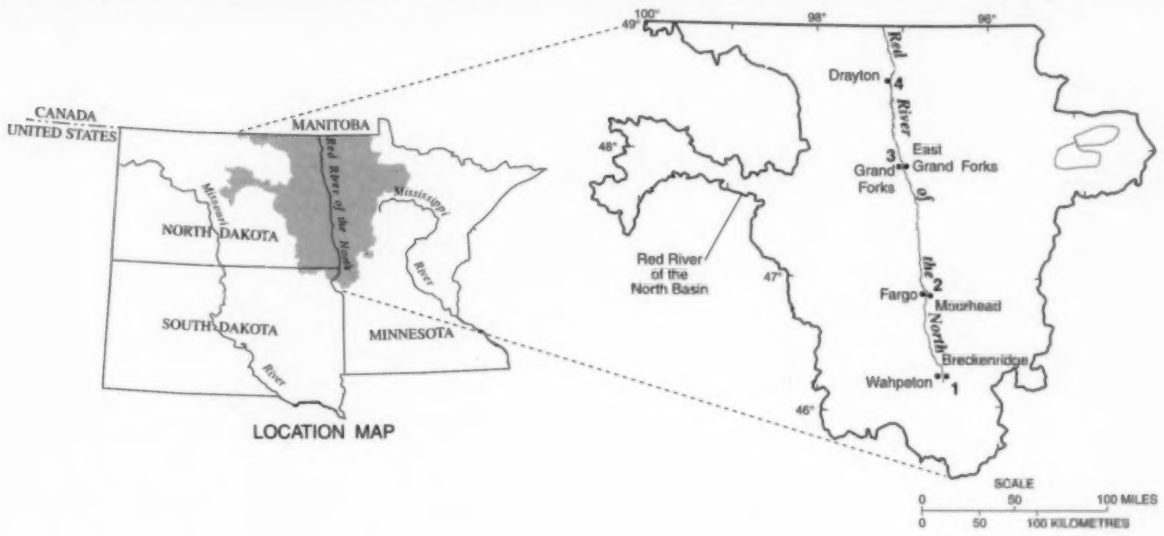
Fig. 1. Sampling locations in the Red River of the North. Carp were collected at sites 1–4; channel catfish were collected at site 2.



unidirectional. Fish exposed to methylmercury in laboratory tests at first had greater concentrations in the blood, spleen, kidney, and liver than in muscle (McKim et al. 1976). Harrison and Klaverkamp (1990) found that after exposure was discontinued, the mercury concentration increased in muscle while decreasing in other organs and tissues. Most of the mercury eventually resides in muscle, although depending upon exposure duration, concentrations may be greater in other tissues (McKim et al. 1976; Huckabee et al. 1979; Harrison and Klaverkamp 1990).

Concentrations of mercury in various tissues do not appear to remain constant through time and may depend on the amount and duration of exposure, although some type of equilibrium may be reached. Concentrations in muscle and liver were highly correlated in a study of fish from the Mississippi River (Wiener et al. 1984). Numerous analyses of freshwater fish tissues have been made (Spry and Wiener 1991; Wiener and Spry 1996), although comparisons of mercury concentrations in selected tissues (liver, muscle, whole body, etc.) of fish are somewhat limited in number and tend to emphasize high concentrations from fish toxicity studies (Matida et al. 1971; Scherer et al. 1975; McKim et al. 1976; Niimi and Kissoon 1994). Data from the literature indicate that when mercury concentrations in fish muscle or whole bodies are low (less than approximately 0.5 μg/g), mercury concentrations in muscle (fillet) tissue are about twice those in liver tissue (Ray et al. 1984; Wiener et al. 1984; Barak and Mason 1990*a*, 1990*b*). When higher whole body or muscle concentrations of mercury are reached (greater than approximately 1 μg/g), this ratio is reversed, and mercury concentrations in livers will be several times higher than whole body concentrations. This same pattern also appears to hold for marine fish (e.g., Al-Hashimi and Al-Zorba 1991; Hornung et al. 1993). Accordingly, reported ranges and standard errors of mercury concentrations in fish muscle or whole bodies are often smaller than those for fish livers, particularly when a few fish samples have high mercury concentrations. These observations imply that fish can tolerate low concentrations of mercury (less than 0.5 μg/g), but when tissue concentrations exceed approximately 1.0 μg/g, mercury is redistributed from the muscle, thereby increasing the mercury burden in the liver. Jernelov and Lann (1971) used the ratio of mercury in the liver to muscle to indicate whether total mercury content was increasing or decreasing in pike (*Esox lucius*). They found that liver to muscle mercury ratios of 1 indicated increasing mercury content while ratios of 0.5 indicated decreasing content, and ratios in between were inconclusive.

This study was conducted as part of the National Water-Quality Assessment Program of the U.S. Geological Survey (Leahy et al. 1990) in the Red River of the North Basin (Fig. 1; Stoner 1991). The purposes of this study were to (*i*) compare mercury concentrations in two tissue types (liver and muscle) and in whole bodies, (*ii*) determine differences in mercury concentrations in the two tissue types and the whole bodies between two size groups of fish, (*iii*) determine if analysis of composite samples accurately reflects the component parts, and (*iv*) to examine the ratios of mercury concentrations in livers, muscle, and whole bodies of two species of different trophic groups.

Methods

Sampling

Carp (*Cyprinus carpio*) were selected for analysis because this species (*i*) was a target taxon for the National Water-Quality Assessment Program, (*ii*) had been the subject of previous mercury analyses in the Red River of the North (Schmitt and Brumbaugh 1990; Minnesota Department of Health 1994; R.M. Goldstein, unpublished data), and (*iii*) was abundant in the study area. Carp were collected by electrofishing during September 1994 at four sites along the main stem of the Red River of the North from its formation at Wahpeton, North Dakota, and Breckenridge, Minnesota, to the Canadian border (Fig. 1). Channel catfish (*Ictalurus punctatus*) were also collected at

Table 1. Lengths (mm) and weights (g) of carp and channel catfish from the Red River of the North by size group and location.

Location	Size group	Length	Weight
Carp			
Wahpeton, N.D., and Breckenridge, Minn.	Large	518±21	1820±279
	Small	464±24	1327±238
Fargo, N.D., and Moorhead, Minn.	Large	550±30	1906±310
	Small	412±9	972±80
Grand Forks, N.D., and East Grand Forks, Minn.	Large	517±18	1762±193
	Small	448±17	1202±71
Drayton, N.D.	Large	542±31	1993±357
	Small	456±30	1215±223
All locations	Large	532±28	1870±289
	Small	445±29	1179±208
Channel catfish			
Fargo, N.D., and Moorhead, Minn.	Large	405±45	528±204
	Small	330±23	245±54

Note: Data are given as the mean ± SD.

one site (Fargo, North Dakota – Moorhead, Minnesota) because they are an important sport fish in the Red River of the North. All fish collected were maintained alive in native water until processing. Each fish was measured to the nearest millimetre (total length), weighed to the nearest gram, and sexed. Fish from each sampling location were split into two size groups: small and large, with seven individuals per group (Table 1). The two size groups of carp and channel catfish were different ($p < 0.001$), for both length and weight. However, the size groups were not similar among locations, i.e., small carp from Fargo were smaller than small carp from the other three sites.

A portion of the liver (ca. 10 g) was removed from each fish and placed in a plastic self-sealing bag. Next, a piece of skin approximately 25 mm square was cut away from the underlying skeletal muscle just below the dorsal fin and folded down to expose the muscle tissue. The muscle tissue (ca. 10 g) below the flap of skin was excised down to the spine (as would occur during filleting) and placed in a self-sealing plastic bag. The remainder of the whole body was wrapped in aluminum foil. All samples were labeled and immediately put on dry ice. Details of the protocol including cleaning procedures, handling techniques, sample identification forms, and quality assurance for data collection were presented by Crawford and Luoma (1993). Frozen samples were shipped within 48 h to the analytical laboratory for analysis.

Composite samples were made for each species by size group. These composites were made by combining approximately equal proportions of the seven individual whole body samples, which had been homogenized, and mixing the combined material (rehomogenizing). Liver and the small muscle portions were not included in the composite sample because they were small relative to whole body weights. Exclusion of the liver and muscle portions was considered insignificant (Wiener et al. 1984). The composite samples were analyzed in the same manner as all other samples.

Analysis

Samples were analyzed at the University of Missouri Environmental Trace Substances Research Center. Samples were homogenized, frozen, and freeze dried. Moisture content was determined as the loss of mass during freeze drying.

Tissue aliquots (ca. 0.5 g) were refluxed for 2 h in 5 mL concentrated nitric acid. Digests were then transferred to volumetric flasks, diluted to 50 mL with 1% (v/v) HCl, and transferred to 30-mL flint glass bottles until analysis. Digested subsamples were amended with stannous chloride and stirred to generate elemental mercury, which was determined by cold vapor atomic absorption using a Perkin-Elmer 3100 AA.[2] Mercury concentrations were reported as micrograms per gram of dry weight, and these values were then converted to wet weight using the moisture content data.

Quality assurance

Moisture determinations were assessed by 24 sets of duplicates, which generally agreed within 2%; 3 sets had greater percent differences (2.6–8.2%). Instruments were calibrated for mercury analyses with at least five standards in the 0–10 µg/L range; correlation coefficients greater than 0.999 were deemed acceptable. A standard was analyzed every 10 samples to check for instrument drift. Reproducibility was examined by 25 duplicate analyses, which agreed within 0–5.9% (mean difference 1.3%). Thirteen analyses of NIST (National Institute for Standards and Technology, Gaithersburg, Md.) oyster tissue (NBS 1566A) yielded a mean concentration of 0.073 ± 0.0025 µg/g (mean ± standard deviation (SD)); all analyses were within 2 SDs of the expected value (0.0642 ± 0.0067 µg/g). Spike recoveries were assessed on 18 samples, which were amended with 0.25 mg Hg. Spike recovery averaged 94% (range 71–114%, SD 12.1%).

Data analysis

The experimental design for carp included two different types of tissue and whole bodies, two size groups, and four locations. Inspection of the mercury concentrations in carp indicated a log-normal distribution, which has been reported for other trace elements (Giesy and Wiener 1977). All data were transformed by using natural logarithms. Two sets of analysis of variance (ANOVA) were used in analyzing the data. The first was a three-factor factorial design to compare concentrations of mercury in the two tissues and whole bodies. Only two-factor interaction effects were tested in this analysis (Cochran and Cox 1957). The three-factor interaction was considered uninterpretable because the whole body included both muscle and liver and so was grouped with the error term. The second was an ANOVA on each tissue type and the whole bodies to determine if differences existed by size group and location. ANOVA was used to compare tissues and whole bodies and size groups in channel catfish from the single collection site. Relations among concentrations in the three body compartments were examined by regression analysis, as were comparisons between species. The regression model used was a random effects model II regression because all concentration data have associated measurement errors. With random effects regressions, dependent and independent variables may be reversed with a consequential change to the resulting slope but not the correlation. The regression line and equation produced with the random effects model is a geometric mean functional regression line referred to as the line of organic correlation (Helsel and Hirsch 1992). The line is the best fit to the data regardless of which variable is considered dependent or independent. The random effects model focuses on the relationship between the two variables but may be of limited predictive application. The relations between mercury concentration and fish size were also examined with regression analysis but in this case, fixed effects model I regressions were used. Data were analyzed using SAS software (release 6.07, SAS Institute Inc., Cary, N.C.).

Results from the composite samples were compared with their components by calculating whole body concentrations of individual fish and then producing a weighted average concentration that was compared with the composite sample concentration. Liver and the small muscle portion were considered to be insignificant in the

[2] The use of brand or trade names in this paper is for identification purposes only, and does not constitute endorsement by the U.S. Geological Survey.

Table 2. Mercury concentrations, in micrograms per gram wet weight, in muscle, whole bodies, and livers of carp from the Red River of the North.

Location	Size group	*n*	Muscle	Whole body	Liver
Wahpeton, N.D., and Breckenridge, Minn.	Large	7	0.35±0.15	0.16±0.05	0.12±0.07
	Small	7	0.33±0.10	0.20±0.07	0.09±0.04
Fargo, N.D., and Moorhead, Minn.	Large	7	0.30±0.05	0.19±0.03	0.12±0.04
	Small	7	0.24±0.03	0.15±0.02	0.07±0.03
Grand Forks, N.D., and East Grand Forks, Minn.	Large	7	0.38±0.09	0.19±0.05	0.16±0.09
	Small	7	0.31±0.07	0.19±0.03	0.09±0.02
Drayton, N.D.	Large	7	0.32±0.06	0.17±0.03	0.16±0.04
	Small	7	0.30±0.07	0.17±0.03	0.10±0.03
All locations	Large	28	0.34±0.09	0.18±0.04	0.14±0.06
	Small	28	0.29±0.08	0.18±0.04	0.09±0.03
Total		56	0.31±0.10	0.18±0.04	0.11±0.06

Note: Data are given as the mean ±SD.

Table 3. Mercury concentrations, in micrograms per gram wet weight, in muscle, whole bodies, and livers of channel catfish from the Red River of the North.

Location	Size group	*n*	Muscle	Whole body	Liver
Fargo, N.D., and Moorhead, Minn.	Large	7	0.16±0.01	0.11±0.02	0.17±0.01
	Small	7	0.19±0.04	0.11±0.03	0.16±0.04
Total		14	0.18±0.04	0.11±0.02	0.16±0.03

Note: Data are given as the mean ± SD.

calculation (Wiener et al. 1984). The mercury concentrations determined for the seven individual whole bodies within a size group were used to calculate total body burdens for each fish. The total body burdens and weights of the seven individuals in a size group were then combined and a composite sample concentration was calculated. The two resultant concentrations, one from a physically produced composite and one from a mathematically produced composite, were compared to determine whether composite samples accurately reflected the combination of the individual components.

Results

ANOVA indicated that mercury concentrations were different in at least one of the three body compartments in both species (carp, $p < 0.0001$; channel catfish, $p < 0.001$). Comparison of mean values (Table 2) for carp (Duncan's multiple range test, $\alpha = 0.05$) indicated that muscle contained the highest concentrations of mercury (0.31 µg/g), followed by whole bodies (0.18 µg/g) and liver (0.11 µg/g). Large carp generally had concentrations of mercury higher than or the same as those of small carp for each of the two tissue types and whole bodies. Sampling location was not a significant factor. Mercury concentrations in tissue compartments were similar throughout the Red River of the North. In channel catfish (Table 3) the muscle and liver contained similar concentrations of mercury (mean = 0.18 and 0.16 µg/g, respectively); both contained higher concentrations than whole bodies (mean = 0.11 µg/g).

Linear regression of natural log transformed mercury concentrations in whole bodies and livers of carp and channel catfish (Fig. 2) indicated that there were significant relationships, although moderate ones, between the concentration of mercury in the liver and the whole body of both carp ($p = 0.001$) and channel catfish ($p = 0.004$); coefficients of determination (R^2 values) were 0.31 for carp and 0.52 for channel catfish. The relationship between the concentrations of

Fig. 2. Plots of $\log_e$ transformed mercury concentrations (µg/g wet weight) in whole bodies (y) and liver tissue (x) of carp and channel catfish from the Red River of the North. The following relations were determined: carp, $y = -0.63 + 0.49x$, $R^2 = 0.31$, $n = 56$; channel catfish, $y = -0.14 + 1.13x$, $R^2 = 0.52$, $n = 14$.

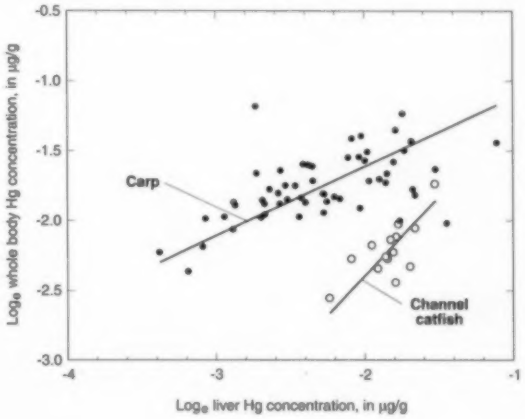


Fig. 3. Plots of $\log_e$ transformed mercury concentrations (μg/g wet weight) in muscle (y) and liver tissue (x) of carp and channel catfish from the Red River of the North. The following relations were determined: carp, $y = 0.11 + 0.57x$, $R^2 = 0.63$, $n = 56$; channel catfish, $y = 0.27 + 1.1x$, $R^2 = 0.26$, $n = 14$. The regression for channel catfish is marginally significant (i.e., slope not equal to zero; $p = 0.06$).

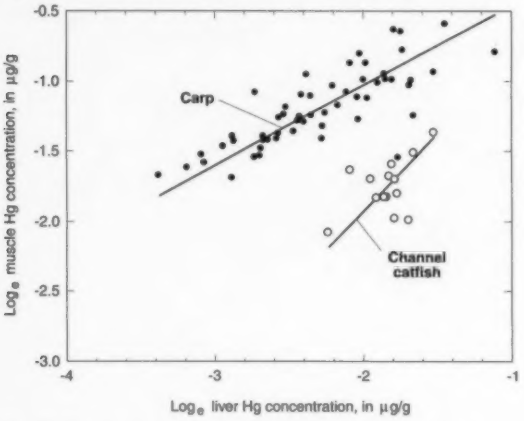
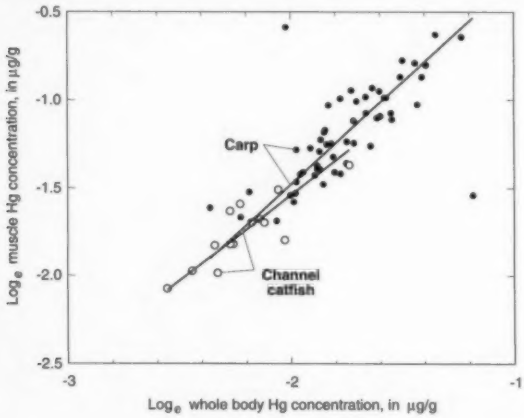


Fig. 4. Plots of $\log_e$ transformed mercury concentrations in muscle (y) and whole bodies (x) of carp and channel catfish from the Red River of the North. The following relations were determined: carp, $y = 0.85 + 1.17x$, $R^2 = 0.46$, $n = 54$ (two outliers not included); channel catfish, $y = 0.41 + 0.97x$, $R^2 = 0.72$, $n = 14$.



mercury in liver and whole bodies was different for carp than for channel catfish. The slopes of the two regression lines were not the same ($p < 0.01$), and the ratio of whole body concentrations to liver concentration was greater in channel catfish than in carp.

The relation between mercury concentrations in liver and muscle was relatively strong in carp ($p = 0.001$, $R^2 = 0.63$) but not in channel catfish ($p = 0.06$, $R^2 = 0.26$) (Fig. 3). The slopes of the two regression lines were similar, although for a given concentration of mercury in the muscle, catfish tended to have higher concentrations of mercury in liver tissue than carp.

The relation between mercury concentrations in whole bodies and muscle appeared to be weaker in carp ($p = 0.0001$, $R^2 = 0.46$) than in channel catfish ($p = 0.0001$, $R^2 = 0.72$). The two outliers (Fig. 4), although shown, were not included in the analysis. The slopes of the two regressions were not different.

Even though mercury concentrations differed between the two size groups of carp, there was no difference between size

Fig. 5. Plots of $\log_e$ transformed mercury concentrations (μg/g wet weight) versus fish weight for liver tissue (A), muscle (B), and whole bodies (C) of carp and channel catfish from the Red River of the North. Linear regression lines are shown only where significant (i.e., slope not equal to zero): for carp livers, $p = 0.001$; for carp muscle, $p = 0.08$.

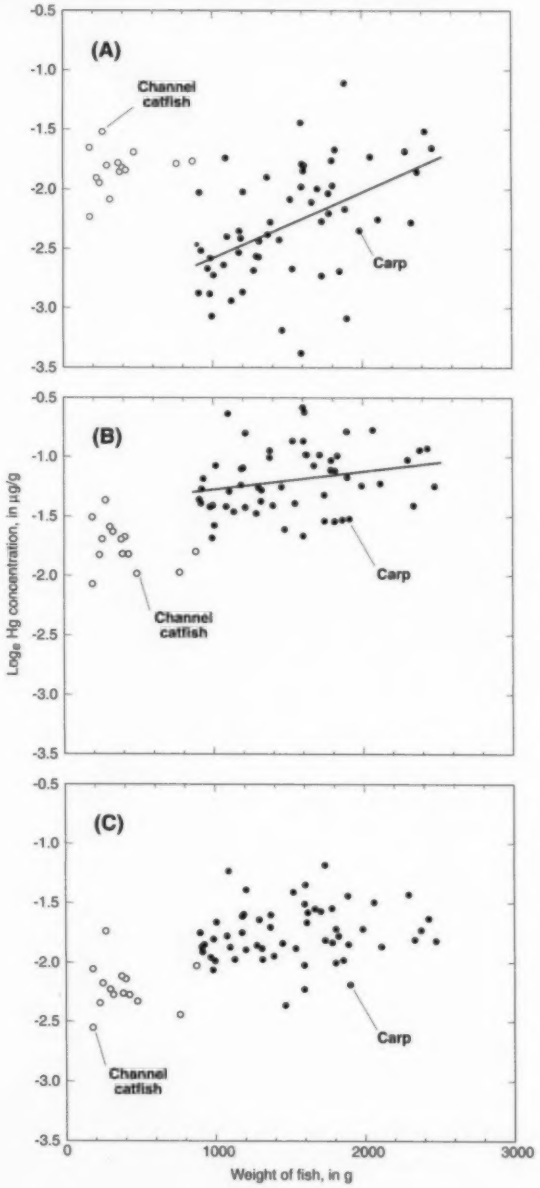


Table 4. Mercury concentrations, in micrograms per gram wet weight, in samples made by compositing seven individuals compared with composites mathematically calculated from the individual component fish.

	Carp								Channel catfish	
Physical composite	0.188	0.144	0.219	0.186	0.176	0.167	0.146	0.209	0.110	0.111
Mathematical composite	0.190	0.145	0.190	0.192	0.171	0.174	0.158	0.208	0.109	0.116
Percent difference (worst case)[a]	1.2	0.4	15.3	3.2	3.1	3.8	8.1	0.7	0.3	3.6

Note: Calculated values are the sum of the product of concentration and fish weight, divided by the sum of fish weights for each group of fish; the last digit, although used for calculations, is not significant.

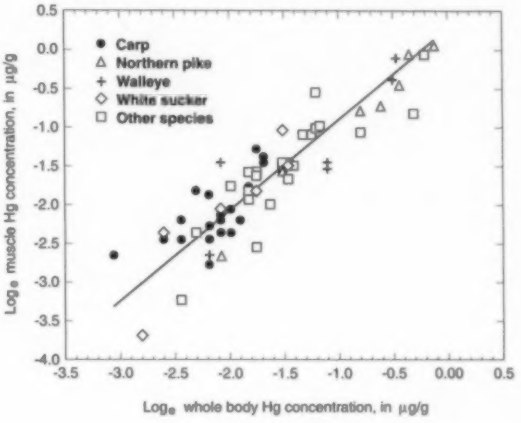
[a]Worst case percent difference is based on the smaller of the two values.

groups of channel catfish. Mercury concentrations in all body compartments tended to increase with size (as measured by total fish weight) in carp but not in channel catfish (Fig. 5). However, the tendency was not strong; only the regression of mercury concentration in liver tissue versus fish weight was significant (i.e., slope not equal to zero, $p = 0.001$, $R^2 = 0.24$; Fig. 5A). It is possible that a greater range in channel catfish sizes would yield significant relations for that species.

Analyses of actual composited samples were compared with mathematical composites by using a nonparametric sign test (Helsel and Hirsch 1992). No significant difference ($p = 0.377$) in the concentrations of mercury was indicated. In most comparisons, the difference between the two concentration values was less than 10% (Table 4).

Mercury concentrations in carp muscle were generally twice the concentrations found in liver tissue (Table 2). While this ratio has been reported in other species (Ray et al. 1984; Wiener et al. 1984; Barak and Mason 1990*a*, 1990*b*), it did not occur in channel catfish. Muscle and liver tissue in channel catfish had similar concentrations of mercury: a ratio approximating 1 (Table 3).

Fig. 6. Plot of $\log_e$ transformed mercury concentrations (µg/g wet weight) in muscle (y) and whole bodies (x) of carp, northern pike, walleye, white sucker, and other species from Minnesota. The following relation was determined (all species combined): $y = 0.35 + 0.92x$, $R^2 = 0.82$, $n = 65$.



Discussion

Many state and provincial programs for determining consumption advisories analyze for muscle (fillet) concentration. Studies to estimate health effects on a population of fish focus on specific organs or whole body concentrations. Relatively few programs have analyzed mercury content in two or more body compartments or tissue types (i.e., muscle, liver, and whole body) from the same fish samples.

The ratio of muscle to whole body mercury concentrations appears to be fairly consistent in many species of freshwater fish from uncontaminated areas. The consistency of the ratio of mercury in muscle versus whole bodies of carp and channel catfish from the Red River of the North (Fig. 4) may be extended to other species as well. Data on mercury concentrations in muscle and whole fish from various waters across Minnesota were obtained from the Minnesota Pollution Control Agency (MPCA). Regression analysis of these data for carp, northern pike, walleye (*Stizostedion vitreum*), white sucker (*Catostomus commersoni*), and 14 other species (only 1–4 samples each) resulted in a significant correlation ($p = 0.0001$, $R^2 = 0.82$) between the concentration of mercury in muscle (fillets with skin on) and whole bodies (Fig. 6). The slope of this regression was not different from the slopes of the regression lines determined for carp and channel catfish (Fig. 4). The MPCA data include fish of different trophic groups, including benthic insectivores, omnivores, and piscivores. Trophic level determines, to a certain extent, the source of mercury contamination as well as the relative amount of methylmercury ingested compared with inorganic mercury (Hildebrand et al. 1980; Philips et al. 1980; MacCrimmon et al. 1983). Because concentrations of mercury in muscle and whole bodies of fish from different trophic groups and different levels of exposure to mercury contamination from across Minnesota remain relatively constant, this ratio may be applicable on a much wider geographical basis. Given the consistency of this relationship, whole body mercury concentration data may be of value in mercury-exposure assessments, even when the focus is on concentrations in muscle.

The ratios of mercury concentrations in livers to the concentrations in either muscle or whole bodies were not as consistent as the ratio between muscle and whole bodies. In carp, livers contained the least concentration of mercury whereas in channel catfish, the concentrations of mercury in livers were similar to those in muscle. This implies a difference in the way mercury (or the organic matrix containing methylmercury) is either processed or stored in liver tissue between these two species. Morphologically, the livers are different between these species. The carp liver is a large, lobed, elongate organ with the posterior portion attached to the intestine for some length (at least 0.5 m in the carp observed in this study). The carp liver is also combined with the pancreas, forming the hepatopancreas (Hoar et al. 1979). The liver of channel catfish

Table 5. Ratio of mean concentrations, in micrograms per gram wet weight, of total mercury in liver and muscle of fish of different trophic groups.

Species	Trophic group	Mercury concentration		Ratio	Source
		Liver	Muscle		
Channel catfish	Piscivore	0.16	0.18	0.89	Present study
Striped bass *(Morone saxatilis)*	Piscivore	0.79	0.77	1.02	Ray et al. 1984
		0.27	0.51	0.53	Ray et al. 1984
Striped bass		0.55	0.35	1.57	Heit 1979
Pike	Piscivore	0.11	0.14	0.78	Barak and Mason 1990*b*
Pike		0.94	0.63	1.49	Koli et al. 1977
Bowfin (*Amia calva*)	Piscivore	0.83	0.63	1.32	Koli et al. 1977
Carp	Omnivore	0.11	0.31	0.35	Present study
White sucker	Insectivore	0.04	0.12	0.33	Harrison and Klaverkamp 1990
Dace (*Leuciscus leucisus*)	Insectivore	0.06	0.14	0.43	Barak and Mason 1990*b*
Perch (*Perca fluviatilis*)	Insectivore	0.08	0.13	0.61	Barak and Mason 1990*b*
Chub (*Leuciscus cephalus*)	Insectivore	0.11	0.17	0.64	Barak and Mason 1990*b*
Tench (*Tinca tinca*)	Insectivore	0.06	0.15	0.40	Barak and Mason 1990*b*
Eel (*Anguilla anguilla*)	Omnivore	0.09	0.21	0.43	Barak and Mason 1990*a*
		0.16	0.34	0.47	
Roach (*Rutilus rutilus*)	Insectivore	0.06	0.13	0.46	Barak and Mason 1990*a*

Note: Data are from field studies.

Table 6. Ratio of mean concentrations of total mercury in liver and muscle or whole bodies of fish of different trophic groups.

Species	Trophic group	Mercury concentration		Ratio	Source
		Liver	Muscle or whole body		
Walleye	Piscivore	6–14	5–8	1.5	Scherer et al. 1975
		18–50	15–45	1.1	Scherer et al. 1975
Rainbow trout (*Oncorhynchus mykiss*)	Insectivore	26–68	20–28	1.9	Matida et al. 1971
		32–114	9–52	2.4	Niimi and Kissoon 1994
Brook trout (*Salvelinus fontinalis*)	Insectivore	58	24	2.4	McKim et al. 1976
		24	10	2.4	McKim et al. 1976
		8	5	1.6	McKim et al. 1976
Carp	Omnivore	1.1[a]	0.45[a]	2.4[a]	Wiener et al. 1984

Note: Data are from laboratory studies or field studies of highly contaminated areas. Concentrations are given as micrograms per gram wet weight unless noted otherwise.

[a]Concentrations are given as micrograms per gram dry weight.

is a smaller, more compact, lobed organ closely associated with the stomach and pyloric caeca, the site of fat digestion. A major difference between the two species is their trophic level. As a piscivore, channel catfish would consume food items much richer in lipids, both in quantity and quality, than the carp, which is a benthic insectivore or omnivore. The liver of channel catfish, or any piscivore, may have a much greater capacity for storing lipid-soluble methylmercury than the livers of nonpiscivores. A review of selected literature indicates that piscivores tend to have a higher ratio of total mercury in the liver than muscle or whole body, compared with nonpiscivorous species. A listing of species and the ratios of mercury in the liver compared with the whole fish or muscle tissue (Table 5) indicates that in nonpiscivores the ratio is approximately one half, while in piscivores the ratio is near one. These ratios can be greatly changed by exposure to high concentrations of mercury in either food or water (Table 6).

If the ratio of mercury concentration in liver to muscle or liver to whole body varies with trophic group, one might expect the ratio of mercury concentration in muscle to whole body to vary as well because of the involvement of the liver in redistribution of mercury in addition to being part of the whole body sample. Although data from Table 5 support this supposition, the regression presented in Fig. 6 does not. It may be that the power of the statistical regression was insufficient to determine a difference or the data transformation may have confounded a difference. In either case, the constancy of the ratios of mercury in the various body compartments should be further investigated and verified across and among trophic groups.

No locational differences in mercury concentrations were discernible in carp along the Red River of the North. It is likely that diffuse rather than large point sources of mercury are dominant in this river. Where major point sources have existed

along rivers, elevated mercury concentrations have been measured in fish downstream (Parks et al. 1991). In areas lacking major point sources, atmospheric deposition of mercury, from both natural and anthropogenic origin, is thought to be the major source. Increases in atmospheric mercury deposition have occurred in the region over the last century, as inferred from analyses of sediment cores from lakes in undisturbed watersheds (Swain et al. 1992). Some fraction (about 8–26%) of the mercury deposited in relatively undisturbed catchments is transported to watercourses (Mierle 1990; Aastrup et al. 1991; Swain et al. 1992). Such transport may be substantially greater in agricultural catchments, such as the Red River of the North Basin. Furthermore, the historical profile may indicate maximal transport around the time of initial tillage of agricultural lands (Brigham 1992), although relatively few data exist to confirm this.

ANOVA indicated a significant difference in mercury concentration between size groups of carp but not channel catfish. The regressions of mercury concentrations in the various tissues versus the weight of the fish (Fig. 5) were for the most part not significant, which is contrary to other studies (e.g., Scott and Armstrong 1972). The range of lengths and weights of carp and channel catfish used in this study was limited and highly variable within size groups, particularly for the channel catfish. Figure 5 shows that most of the fish sizes grouped together except for two channel catfish in the large size group. The lack of a trend of increasing mercury concentration with size in channel catfish observed in this study may be due to three factors, (*i*) the limited sample size, (*ii*) the small range of sizes sampled, or (*iii*) the variability of the sizes of the individuals both between and within size groups.

Although whole body concentrations of mercury were similar between large and small carp, small carp had lower concentrations in both muscle and liver than large carp. This implies that some compartment(s) not analyzed in small carp has (have) a higher concentration of mercury than in large carp such that the whole body concentrations become similar. Mercury also accumulates in blood, spleen, brain, kidney, and other tissues. Whether that accumulation is differential between differently sized fish is uncertain. Alternatively, the mathematics of dilution of mercury during homogenization of the whole bodies of larger fish with a larger amount of noncontributing material (e.g., bone and scales) may have reduced the whole body concentration sufficiently to match that of the smaller carp. The latter alternative seems the more probable, although additional analyses of different size groups that include a more complete range of tissue types would certainly contribute to the clarification of this seeming contradiction.

Even though composite samples have been used for determining average contaminant concentrations in fish, there has been little study to verify the accuracy of composites. While statistical theory predicts the power of using a composite sample (Tetra Tech Inc. 1987), rigorous field testing has not been done. The comparison of the analytical results from composite samples with those from mathematically derived composite samples consisting of the individual parts indicates that composite samples accurately reflect the mean concentration of contaminants from the individuals and can be a cost effective and statistically effective technique for determining average contaminant concentrations. When monitoring programs rely on physical composites, it also may be desirable to analyze a subset of individual fish to obtain an estimate of the variability associated with the individuals that comprise a composite. Standard deviations for several carp and channel catfish samples from this study are included in Tables 2 and 3. These values may provide error estimates for other analyses where the concentrations are similar in magnitude, but the variance of the individuals contributing to the composite is unknown.

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References

Aastrup, M., Johnson, J., Bringmark, E., Brinmark, I., and Iverfeldt, A. 1991. Occurrence and transport of mercury within a small catchment area. Water Air Soil Pollut. **56**: 155–167.

Al-Hashimi, A.H., and Al-Zorba, M.A. 1991. Mercury in some commercial fish from Kuwait: a pilot study. Sci. Total Environ. **106**: 71–82.

Barak, N.A.E., and Mason, C.F. 1990*a*. Mercury, cadmium and lead in eels and roach: the effects of size, season and locality on metal concentrations in flesh and liver. Sci. Total Environ. **106**: 249–256.

Barak, N.A.E., and Mason, C.F. 1990*b*. Mercury, cadmium and lead concentrations in five species of freshwater fish from eastern England. Sci. Total Environ. **106**: 257–263.

Bloom, N.S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquat. Sci. **49**: 1010–1017.

Brigham, M.E. 1992. Accumulation of mercury in Minnesota, Alaska, and Wisconsin lakes. M.S. thesis, University of Minnesota, Minneapolis, Minn.

Cochran, W.G., and Cox, G.M. 1957. Experimental designs. 2nd ed. John Wiley and Sons, New York.

Cope, W.G., Wiener, J.G., and Rada, R.G. 1990. Mercury accumulation in yellow perch in Wisconsin seepage lakes: relation to lake characteristics. Environ. Toxicol. Chem. **9**: 931–940.

Crawford, J.K., and Luoma, S.N. 1993. Guidelines of studies of contaminants in biological tissues for the National Water-Quality Assessment Program. Open File Rep. U.S. Geol. Surv. No. 92-494.

Eisler, R. 1987. Mercury hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish Wildl. Serv. Biol. Rep. No. 85(1.10), Contaminant Hazard Reviews Rep. No. 10.

Giesy, J.P., Jr., and Wiener, J.G. 1977. Frequency distributions of trace metal concentrations in five freshwater fishes. Trans. Am. Fish. Soc. **106**: 393–403.

Harrison, S.E., and Klaverkamp, J.F. 1990. Metal contamination in liver and muscle of northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) and in sediments from lakes near the smelter at Flin Flon, Manitoba. Environ. Toxicol. Chem. **9**: 941–956.

Heit, M. 1979. Variability of the concentrations of seventeen trace elements in the muscle and liver of single striped bass, *Morone saxatilis*. Bull. Environ. Contam. Toxicol. **23**: 1–5.

Helsel, D.R., and Hirsch, R.M. 1992. Statistical methods in water resources. Stud. Environ. Sci. **49**.

Hildebrand, S.G., Strand, R.H., and Huckabee, J.W. 1980. Mercury accumulation in fish and invertebrates of the North Fork Holston River, Virginia and Tennessee. J. Environ. Qual. **9**: 393–400.

Hoar, W.S., Randall, D.J., and Brett, J.R. 1979. Fish physiology. Vol. VIII. Bioenergetics and growth. Academic Press, New York.

Hornung, H., Krom, M.D., Cohen, Y., and Bernhard, M. 1993. Trace metal content in deep-water sharks from the eastern Mediterranean Sea. Mar. Biol. (Berlin), **115**: 331–338.

Huckabee, J.W., Elwood, J.W., and Hildebrand, S.G. 1979. Accumulation of mercury in freshwater biota. *In* The biogeochemistry of mercury in the environment. *Edited by* J.O. Nriagu. Elsevier/North-Holland Biomedical Press, New York. pp. 277–302.

Jernelov, A., and Lann, H. 1971. Mercury accumulation in food chains. Oikos, **22**: 403–406.

Koli, A.K., Williams, W.R., McClary, E.B., Wright, E.L., and Burrell, T.M. 1977. Mercury levels in freshwater fish of the State of South Carolina. Bull. Environ. Contam. Toxicol. **17**: 82–89.

Leahy, P.P., Rosenshein, J.S., and Knopman, D.S. 1990. Implementation plan for the National Water-Quality Assessment program. Open File Rep. U.S. Geol. Surv. No. 90-174.

MacCrimmon, H.R., Wren, C.D., and Gots, B.L. 1983. Mercury uptake by lake trout, *Salvelinus namaycush*, relative to age, growth, and diet in Tadenac Lake with comparative data from other Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. **40**: 114–120.

Matida, Y., Kumada, H., Kimura, Y., Saiga, Y., Nose, T., Yokote, M., and Kawatsu, H. 1971. Toxicity of mercury compounds to aquatic organisms and accumulation of the compounds by the organisms. Bull. Freshwater Fish. Res. Lab. (Tokyo), **21**: 197–227.

McKim, J.M., Olson, G.F., Holcombe, G.W., and Hunt, E.P. 1976. Long-term effects of methlymercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): toxicity, accumulation, distribution, and elimination. J. Fish. Res. Board Can. **33**: 2726–2739.

Mierle, G. 1990. Aqueous inputs of mercury to precambrian shield lakes in Ontario. Environ. Toxicol. Chem. **9**: 843–852.

Minnesota Department Of Health. 1994. Minnesota fish consumption advisory. Report of the Minnesota Department of Health, Minneapolis, Minn.

Niimi, A.J., and Kissoon, G.P. 1994. Evaluation of the critical body burden concept based on inorganic and organic mercury toxicity to rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol. **26**: 169–178.

Parks, J.W., Curry, C., Romani, D., and Russel, D.D. 1991. Young northern pike, yellow perch and crayfish as bioindicators in a mercury contaminated watercourse. Environ. Monit. Assess. **16**: 39–73.

Phillips, G.R., Lenhart, T.E., and Gregory, R.W. 1980. Relation between trophic position and mercury accumulation among fish from the Tongue River Reservoir, Montana. Environ. Res. **22**: 73–80.

Ray, S., Jessop, B.M., Coffin, J., and Swetnam, D.A. 1984. Mercury and polychlorinated biphenyls in striped bass (*Morone saxatilis*) from two Nova Scotia rivers. Water Air Soil Pollut. **21**: 15–23.

Scherer, E., Armstrong, F.A.J., and Nowak, S.H. 1975. Effects of mercury contaminated diet upon walleyes, *Stizostedion vitreum vitreum* (Mitchill). Can. Fish. Mar. Serv. Resour. Dev. Branch (Winnipeg) Tech. Rep. No. 597.

Schmitt, C.J., and Brumbaugh, W.G. 1990. National Contaminant Biomonitoring Program: concentrations of arsenic, cadmium, copper, lead, mercury, selenium, and zinc in U.S. freshwater fish, 1976–1984. Arch. Environ. Contam. Toxicol. **19**: 731–747.

Scott, D.P., and Armstrong, F.A.J. 1972. Mercury concentration in relation to size in several species of freshwater fishes from Manitoba and northwestern Ontario. J. Fish. Res. Board Can. **29**: 1685–1690.

Spry, D.J., and Wiener, J.G. 1991. Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. Environ. Pollut. **71**: 243–304.

Stoner, J.D. 1991. National Water-Quality Assessment Program: Red River of the North. Open File Rep. U.S. Geol. Surv. No. 91–151.

Swain, E.A., Engstrom, D.R., Brigham, M.E., Henning, T.A., and Brezonik, P.L. 1992. Increasing rates of atmospheric mercury deposition in midcontinental North America. Science (Washington, D.C.), **257**: 784–787.

Tetra Tech Inc. 1987. Bioaccumulation monitoring guidance: strategies for sample replication and compositing. Rep. No. 430/09-87-003. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection, Washington, D.C.

U.S. Environmental Protection Agency. 1989. Assessing human health risks from chemically contaminated fish and shellfish: a guidance manual. Rep. No. 503/8-89-002. U.S. Environmental Protection Agency, Office of Marine and Estuarine Protection and Office of Water Regulations and Standards, Washington, D.C.

U.S. Environmental Protection Agency. 1992. National study of chemical residues in fish. Vols. I and II. Rep. No. 823-R-92-008. U.S. Environmental Protection Agency, Washington, D.C.

Weatherly, A.H., Lakes, P.S., and Rogers, S.C. 1980. Zinc pollution and the ecology of the freshwater environment. *In* Zinc in the environment. Part I. Ecological cycling. *Edited by* J.O. Nriagu. Wiley-Interscience, New York. pp. 337–418.

Wiener, J.G., and Spry, D.J. 1996. Toxicological significance of mercury in freshwater fish. *In* Environmental contaminants in wildlife: interpreting tissue concentrations. *Edited by* W.N. Beyer, G.H. Heinz and A.W. Redmon. Lewis Publishers, Boca Raton, Fla. pp. 299–343.

Wiener, J.G., Jackson, G.A., May, T.W., and Cole, B.P. 1984. Longitudinal distribution of trace elements (As, Cd, Cr, Hg, Pb, and Se) in fishes and sediments in the Upper Mississippi River. *In* Proceedings of the 15th Annual Meeting of the Mississippi River Research Consortium: Contaminants in the Upper Mississippi River, LaCrosse, Wis., April 14–15, 1982. *Edited by* J.G. Weiner, R.V. Anderson, and D.R. McConville. Butterworth Publishers, Boston, Mass. pp. 139–170.

Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea)

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Abstract: *Cucumaria frondosa* was studied in the field and in the laboratory to elucidate its development, growth, substrate selection, and migration patterns. In 1992 and 1993, spawnings in the laboratory and in the lower St. Lawrence Estuary occurred in mid-June. Males spawned first, followed shortly by females. The fertilized eggs and resulting embryos were buoyant. They developed into pentactula 9 d after fertilization. In the laboratory, embryonic development was fastest at 12°C, pH 8, and salinity 26 psu (practical salinity unit). The first contact of embryos with the substrate, made with the tentacles, occurred ca. 48 d after fertilization. Following a search period of 3–40 h, settlement was achieved using the ambulacral podia. Laboratory and field observations showed that gravel or rock were favored by larvae during settlement. After reaching ca. 2.8 mm in length, ca. 4–5 months later, young sea cucumbers moved to sheltered, illuminated areas of rocky substrate, and migrated from protected to exposed areas after they exceeded ca. 35 mm in length. Overall, the growth rates were maximal during the spring and summer phytoplanktonic blooms with rising temperature. Field observations showed a size-dependent migration from photic to aphotic depths (≥40 m depth), when the animals reached sexual maturity.

Résumé : Des études en laboratoire et dans le milieu naturel ont été réalisées pour comprendre le développement, la croissance, le choix d'un substrat et les migrations de *Cucumaria frondosa*. Les pontes de 1992 et 1993 se sont produites à la mi-juin, dans l'estuaire du Saint-Laurent et en laboratoire. Les mâles pondent un peu avant les femelles et leurs oeufs, comme leurs embryons, flottent près de la surface de l'eau. Neuf jours après la fécondation, ils atteignent le stade pentactula. Les meilleures conditions de culture en laboratoire ont été obtenues à une température de 12°C, un pH de 8 et une salinité de 26 psu. Les embryons contactent le substrat pour la première fois par l'intermédiaire des tentacules, 48 jours après la fécondation. La fixation finale à l'aide des podia ambulacraires est complétée après une période exploratoire de 3 à 40 h. Des expériences sur le terrain et en laboratoire montrent que les larves privilégient le gravier et les rochers pour se fixer. Ayant atteint une longeur de ca. 2,8 mm, 4–5 mois plus tard, les jeunes concombres de mer se déplacent vers les zones éclairées et protégées du substrat. Ils ne poursuivent leur migration vers des zones exposées qu'après avoir atteint la taille de ca. 35 mm. Le taux de croissance culmine au moment de la floraison estivale du phytoplancton lors de l'élévation de la température. Des observations effectuées dans la nature ont montré que la migration de la zone euphotique à la zone aphotique (≥40 m de profondeur) est reliée à la taille des individus parvenus à maturité sexuelle.

Introduction

The sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) is one of the most abundant and widely distributed species of echinoderms along the east coast of Canada (Gosner 1979). It can be found throughout the St. Lawrence Estuary and Gulf from the lower tide limit to the deepest waters and in numerous other locations around the North Atlantic. Some aspects of its reproduction and ecology have been studied during the past 85 years (Arts 1910; Runnström 1918; Runnström and Runnström 1919; Thorson 1946; Jordan 1972) but as yet, its life history has not been completely described.

The spawning and development of many other sea cucumber species have been detailed in studies by Chia and Buchanan (1969), Holland (1981), McEuen (1986, 1987), McEuen and Chia (1985, 1991), Cameron and Fankboner (1989), and Dolmatov and Yushin (1993). Still, except for some evidence from the work of Young and Chia (1982) on *Psolus chitonoides*, and that of Bulteel et al. (1992) on *Holothuria tubulosa*, little is known about the substrate selection, recruitment, and migration of young sea cucumbers after settling and about their juvenile life stages. In that regard, holothuroids remain poorly known compared with asteroids and echinoids (Birkeland et al. 1971; Barker 1977; Barker and Nichols 1983; Lucas 1984; Zann et al. 1987; Rowley 1989, 1990; Harrold et al. 1991; Keesing et al. 1993). The growth rates of very few species of sea cucumbers have been modelled (Buchanan 1967; Ebert 1978; Conand 1988) and Rutherford (1973) is the only person to have studied the growth of newly recruited *Cucumaria pseudocurata* over a year. Further, to our knowledge, no study has ever followed the growth rate of a sea

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cucumber from fertilization until the size at sexual maturity in relation to seasonal environmental factors. The only study of that kind in echinoderms is the work of Lucas (1984), who followed the growth and maturation of the sea star *Acanthaster planci* from in vitro fertilization over 8 years.

The present study extends our knowledge of the life history of the sea cucumber *Cucumaria frondosa* by considering its long-term development under natural environmental conditions and provides information on conditions suitable for the culture of embryos and young. Combining field and laboratory data, our research provides new information on spawning behavior, larval settlement preferences, postsettling migration, growth rate, and the time necessary to attain sexual maturity. Finally, this research draws a portrait of the spatial distribution of the individuals well after the first settlement, and describes a large-scale size-dependent migration with depth.

Materials and methods

Collection of individuals

Cucumaria frondosa were dredged at ca. 80 m depth at Sainte-Anne-des-Monts on the south shore of the Lower St. Lawrence Estuary, eastern Canada (48°21′N, 68°47′W), prior to the anticipated breeding season of June, in 1992 and 1993. Around 400 adults ca. 350 mm in length were transferred within 6 h of collection to six tanks (550 L) supplied with slowly running seawater under natural photoperiod.

Spawning and collection of gametes

Collection and handling stimulated 9 of the 178 female individuals to spawn in the laboratory ca. 18 h after capture. No males spawned naturally. Of the ca. 9000 oocytes spawned by each female, ca. 4000 were collected with a glass pipette near the gonopore.

Between 50 and 60 sea cucumbers were randomly taken from the holding aquarium and kept in 4-L beakers, to induce spawning. We tried different methods to obtain female gametes: (*i*) collection directly from the gonad by surgery, (*ii*) injection of either 20 mL of KCl (0.52 M) or 10 mL of 1-methyladenine (10^{-3} M stock solution in distilled water) into the coelomic cavity, (*iii*) induction by thermal shocks, (*iv*) changing light intensity, and finally (*v*) exposing the animals to various concentrations (from 2×10^4 to 2×10^7 cells/mL) of phytoplanktonic cells (*Thalassiosira gravida*, *Skeletonema costatum*) that bloom at the same time as the sea cucumber *Cucumaria frondosa* spawns in the St. Lawrence Estuary (Hamel et al. 1993; Hamel and Mercier 1995).

Development under natural environmental conditions

When obtained, mature eggs (collected from four females) were inseminated with sperm collected surgically from the gonads of 10 males and mixed in 4 L of seawater for a final concentration of 2.5×10^4 spermatozoa/mL. After 3 min of contact in the culture, the residual spermatozoa were removed and the fertilized oocytes were rinsed in seawater (5°C, 28 psu).

A group of fertilized eggs was used to study long-term development under natural environmental conditions (day length, food availability, pH, temperature, and salinity). The eggs were transferred to a tank supplied with running seawater and held under natural conditions for the duration of the experiment (24 months). The experiment was conducted in triplicate. Thirty to forty embryos were collected every 30 s for the first 3 h, every hour for the next 48 h, and finally once a day for the next 2 weeks. Samples were eventually collected once a week after no rapid modification of the embryos was observed. Each embryo was measured live and described under light microscopy with a graduated ocular. A new stage was considered to have been attained when 50–60% of the embryos reached it. Larval behavior, including migration events and buoyancy changes, was also noted.

During this study, the temperature and salinity of the running seawater were recorded continuously while the pH and concentration of chlorophyll *a* were recorded weekly. The temperature was noted on a Peabody Ryan thermograph, the salinity was measured using a salinometer (Auto-Salt 8400), and the pH was measured with an electronic pH meter. The chlorophyll *a* concentration was determined on two 50-mL subsamples of seawater. Samples were filtered through Whatman GF/C filters, which were immediately extracted with 90% acetone for 24 h prior to pigment concentration determination. The data for day length were obtained from the weather station at the Québec airport by Environment Canada, Atmospheric Environment Service.

Influence of pH, salinity, temperature, and gamete concentration on development

Fertilization and development were also tested at different pH values, salinities, and temperatures to determine the best culture conditions for the embryos up to the young pentactula stage. The ranges selected reflect the habitat where the sea cucumbers can be found. In culture, the pH was adjusted by addition of either sodium biphosphate or sodium bicarbonate; salinities were achieved by adding a solution of sea salt (Instant Ocean) dissolved in demineralized water. The oocytes used to assess the effect of salinity on development were always preincubated for 60 min in the test conditions prior to the addition of sperm. Two replicates of all culture conditions were reproduced in 4-L glass jars in which the seawater was continuously aerated, agitated, and renewed every 4 h to prevent modification of the culture. Gross optimal conditions for fertilization and development were determined during a series of preliminary experiments and those first results were used to make a more refined assessment. The most favorable conditions of pH and salinity were determined at the best temperature by measuring fertilization success and the rate of the first few cleavages. These optimal pH and salinity conditions were then used in experiments to determine more precisely the influence of temperature on development. All experiments were conducted under a 12 h light : 12 h dark photoperiod, using uniform gamete ratios (spermatozoa:oocyte ratio of 125:1).

To evaluate the effect of sperm and oocyte concentrations on fertilization rate, different concentrations of sperm, varying from ca. 0.1 to 20×10^4 spermatozoa/mL, were tested for the capacity to induce high fertilization success without polyspermic fertilization. Polyspermic fertilization is defined as two or more spermatozoa penetrating one oocyte. The concentration of sperm was evaluated after dilution in a 4-L beaker by counting spermatozoa (five replicates) with a hemacytometer under light microscopy. Each experiment was conducted with spermatozoa from one male and oocytes from one female, in triplicate, using various concentrations of oocytes (100, 500, 1000, and 1500 per 4-L beaker), representative of the range in natural spawning conditions (Hamel and Mercier 1996). The experiments were conducted under optimal conditions of pH, salinity, and temperature as determined in the preceding steps. The DNA-specific dye Hoechst 33258 was used to determine under fluorescence microscopy (Leitz Diaplan fluorescence microscope) how many male pronuclei were present in the fertilized oocytes.

Larval settlement

On the basis of the methods used by Young and Chia (1982), Bachelet and Butman (1991), and Lambert (1991), the settling preference of sea cucumber pentactulae was observed in the laboratory. The larvae were given a choice between several settling substrates under various experimental conditions described below.

Experimental design

The experiments were conducted in 720 × 720 × 300 mm tanks. Laboratory-reared pentactulae of *Cucumaria frondosa* were given a choice of six sediments: mud (<0.06 mm in diameter), sand (0.5–2 mm in diameter), granules (2–5 mm), small rocks (≈35 × 35 ×

10 mm in size) with either numerous crevices ≤5 mm (R1) or smooth surfaces (R2), and the glass artificial bottom of the tank. Except when otherwise mentioned, all substrates were cleaned and heated in an oven at 500°C for 48 h to destroy all material that might induce a false response because of possible chemical attraction. The tank was filled to a depth of 150 mm and continuously supplied with filtered (5 µm) artificial seawater, at the pH, salinity, and temperature levels previously described to be the best living conditions for the larvae. The various substrates were arranged in a random checkerboard design consisting of 36 boxes, 100 × 100 × 100 mm each. The small tank facilitated uniform and rapid dispersion of the larvae in the water column and optimized the accessibility of all available substrate. All of the experiments were repeated 4 times and were based on 2 weeks of observations. After definitive settlement, each soft substrate was sieved and washed to isolate the larvae, which were then counted. The number of larvae and their relative position on the larger hard substrates were visually assessed. Examination under a stereomicroscope was used to confirm all counts.

Preparation and choice of larvae

Before each experiment, a few individuals from the larval cultures were tested under light microscopy for their ability to settle, as determined by the progressive disappearance of cilia and the appearance of the first two ambulacral podia, which are necessary conditions for definitive settlement. This was done to ensure that the pentactulae were always in the same state prior to all experiments. Generally the larvae were placed in the experimental tank when they were ready to settle but still highly mobile and able to exhibit searching behaviors for another 24–48 h. After their selection, 100 larvae were released randomly into each settling tank.

Influence of current and light

The first experiment compared the distribution of settled larvae on the various substrates with regard to the light conditions. One checkerboard tank was exposed to the photoperiod previously determined to be optimal for the culture of embryos and another was kept in total darkness. In addition, those two conditions were tested in the presence and in the absence of current. The current had a steady unidirectional smooth turbulent flow with a shear velocity of 5.5 cm/s (approximating the current speed observed in the field during the turning tide). As a control, the settlement of dead larvae (killed by thermal shock) was assessed with and without current. We also verified whether the larvae would modify their choice if the light was turned on 12 h after their definitive settlement in total darkness, in both still and turbulent conditions.

Influence of other factors

We conceived another experiment in which the substrates of one tank were covered with mucus from adult sea cucumbers, and the other tank was left as described above. The experiment was conducted in turbulent conditions and under the usual photoperiod. We wanted to verify whether or not the presence of mucus would modify the previously observed substrate selection on the basis of the physical properties of the sediments. Finally, we tried the same experimental procedure with unheated substrates collected directly from the field (covered by various calcareous algae and small encrusting animals), again to determine if factors in the field could modify the choice of the larvae for a particular type of substrate.

Long-term growth of juveniles in the field

The field experiment was conducted at Les Escoumins on the north shore of the St. Lawrence Estuary (eastern Canada, 48°32′N, 69°41′W). This site was chosen because (*i*) it is representative of the rocky subtidal zone of the lower St. Lawrence Estuary and supports a high density of sea cucumbers at all depths, (*ii*) the substrate is a regular bedrock (granite) formation with few small boulders, which facilitated sea cucumber collection, (*iii*) the slope of the substrate is progressive (10–25°), and (*iv*) ice accumulation is minimal so that the site is accessible throughout the year.

Four hundred and fifty individuals (35 mm length) were collected at the study site and placed in 12 mesh boxes (2 × 2 × 1 m; 3 at each depth) for a period of ca. 3 years at 0 (LWST), 5, 10, 15, and 20 m depth where large natural densities of *Cucumaria frondosa* were found. The initial size selected for this experiment corresponds to the maximal size attained during the laboratory experiments. At this size and stage of development, the animals are naturally exposed on the substrate and the transfer into the mesh box was probably less disturbing than for smaller, hidden animals. Every 3 months, the length of all individuals was determined using self-contained underwater breathing apparatus (SCUBA). In addition, five individuals were collected from one of the three mesh boxes placed at each depth, to evaluate the level of maturity by histological methods (Hamel et al. 1993; Hamel and Mercier 1996). The number of gonadal tubules, their length (from the point of attachment to the gonoduct), and the total number of mature oocytes contained in the whole gonad were counted. Temperature was continuously recorded, over the 3-year period, with two Peabody-Ryan thermographs. Day–length variations were also noted and the concentration of chlorophyll *a* was recorded weekly, using previously described methods.

Temporal evolution of spatial distribution in the field

SCUBA dives were conducted once at the beginning of each season, at the same field location, from spring 1992 to winter 1994. Summer observations were made 3 weeks after spawning (June) when settlement occurred. We surveyed a population of sea cucumbers along a 100 × 2 m transect parallel to the coast, from 0 (lower water spring tides; LWST) to 60 m depth. From LWST to 60 m, the bottom is mainly composed of bedrock, boulders, and gravel. Below 60 m, the proportion of muddy and sandy bottom increases and constitutes more than 90% of the substrate. The sea cucumbers' length and sex (visual inspection of the gonopore or gamete collections by a syringe through the body wall) were recorded. Individuals were classified as immature (when no mature gametes were detectable), male, or female. On all collecting dates, the male and female ratios of sea cucumbers were also noted. Finally, we recorded the number of the predacious sea stars *Solaster endeca* and sea urchins *Strongylocentrotus droebachiensis* at each depth.

Results

Sexual dimorphism

Cucumaria frondosa is sexually dimorphic. When the feeding podia around the mouth are extended, it is easy to observe the large gonopore located a few millimetres outside the crown of feeding podia. The female gonopore is a single, large (4 mm), and long (7 mm) papilla with one genital pore on the tip. The male gonopore (8–10 mm wide) is divided into numerous papillae (generally 5–22). This dimorphism allows the sexes to be easily separated when the feeding podia are extended. In very rare cases (1/60 for male, 1/200 for female), more than one gonopore was found on the same individual. In those instances, the second gonopore was posterior (20–40 mm) to the normal one in the same axis along the base of the elongated tissues covering the aquapharyngeal bulb, but it seemed to be not functional. During observed spawning activity, those individuals did not release gametes through this supplementary gonopore.

Collection of gametes

Only freshly spawned oocytes completed germinal vesicle breakdown (GVBD). The passage of eggs through the oviduct

during spawning was apparently necessary for oocyte maturation. When collected surgically, oocytes did not initiate GVBD, and consequently they were nonfertilizable. Artificially triggered spawnings occurred only when individuals were kept in total darkness for a minimum of 8 h and were then suddenly exposed to light in combination with a rapid increase in temperature (from 5 to 10°C in 1 h). This method induced 55% of mature females to spawn in an average of 1.5 h. Male spawning has never been initiated artificially, but spermatozoa collected directly from the gonad were fully effective in fertilizing mature oocytes.

Spawning

We observed spawning events of both male and female *Cucumaria frondosa* on several occasions in the laboratory and in the field in mid-June of 1992 and 1993. Spawning events were preceded by incremental peristalsis of the body wall, initiated from the anus. The contractions had a duration of ca. 20 s over 6 h and were separated by short interruptions (10–15 s). Sixty minutes before spawning, the tentacles were extended, although less than during feeding behavior, and the gametes were released. During female spawning, completed in 3 h, a long (ca. 10 mm) cylindrical mass of oocytes (3 mm wide) was expelled. Each pellet contained at least 200–450 oocytes. The turbulence of the seawater rapidly disrupted the membrane and the oocytes dispersed into the water. The buoyant eggs were found very close to the surface. The mature oocytes are red, 900 ± 200 μm in diameter, and surrounded by a gelatinous coat 45 μm thick. Male spawning behavior was similar to that of the female except that the sperm immediately dispersed into the water, helped by a rapid movement of the tentacles near the gonopore. The sperm head is almost spherical (4 μm in diameter) and the flagellum measures ca. 155 μm. The acrosomal filament measures ca. 110 μm in length when fully extended. During and after spawning, no individual was observed to collect its own gametes (oocytes or spermatozoa) from the water column with the tentacles, although the feeding behavior of adult sea cucumbers for the duration of observations (1 week) continued normally in a water column filled with embryos. This observation was confirmed by examination of intestinal and stomach contents, which showed only the presence of phytoplanktonic cells.

Development under natural environmental conditions

The developmental kinetics, with the corresponding size of the embryos and photographic illustrations of some of the stages, are given in Fig. 1 and Table 1. Five minutes after fertilization, the fertilization membrane elevation was completed. The first and second polar bodies were extruded after 9 and 22 min, respectively. The second polar body was approximately twice the size of the first (15 and 27 μm in diameter, respectively). After fertilization, a unilateral cleavage divided the egg into two equal parts. After completion of this first cleavage along the animal–vegetal axis (equal, holoblastic, and radial), the embryo consisted of two roughly hemispherical blastomeres. The second cleavage occurred again along the animal–vegetal axis, yielding more spherical blastomeres than the preceding stage, and the size of the embryo increased slightly, from 1.30 to 1.35 mm in diameter (Table 1). The plane of the third cleavage was equatorial, giving rise to eight approximately spherical blastomeres. After the eight-cell stage, fragments of the embryo (vacuoles) were released and maintained for a short time in the perivitelline space. These structures disappeared before the 16-cell stage, after which unequal cleavages occurred between the two poles (Fig. 1). From the 16-cell stage to the 128-cell stage, the animal pole developed twice as fast as the vegetal pole (Fig. 1). Each large blastomere observed in the vegetal pole possessed one nucleus when stained with the DNA-specific dye Hoechst 33258. Compaction during early blastula occurred 35 h after fertilization when the space between each blastomere decreased considerably. About 59 h after fertilization, the embryo began to spin in a counterclockwise direction (visualization facilitated by the presence of the blastopore) within the fertilization membrane before hatching.

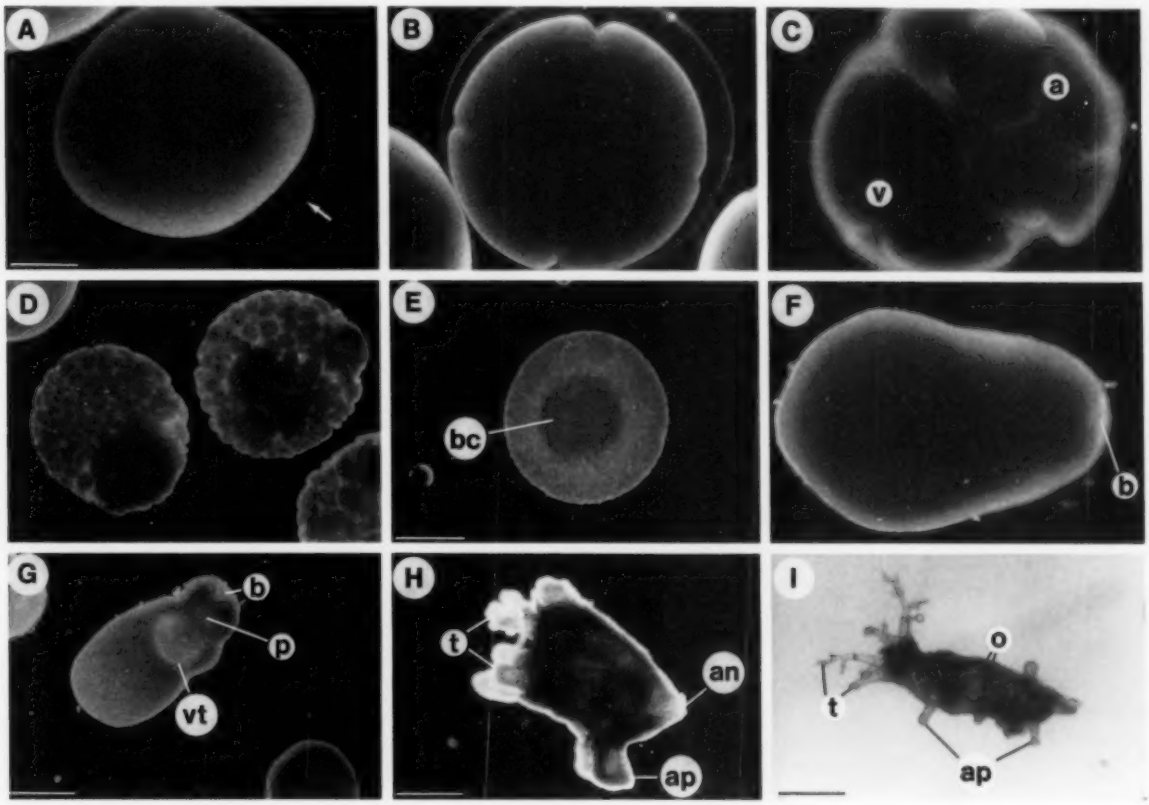
Embryos hatched from the fertilization membrane ca. 62 h after fertilization, as the blastula emerged from a hole that opened on one side of the fertilization membrane. Newly hatched blastulae developed into young gastrulae 10 h later and elongated along the animal–vegetal axis until they were 5- to 6-day-old embryos. These gastrulae swam slowly with the help of the cilia (ca. 43 μm long) covering their entire surface. A small ventral depression, positioned one-third the body length from the apical end, marked the site of the future vestibule. At this time, 7–9 d after fertilization, the embryos were vitellariae (Table 1, Fig. 1). This vitellaria showed a uniform ciliation all around the body. The rudiments of the five primary tentacles appeared in the vestibule ca. 8 d after fertilization and the podial pit was visible (Fig. 1). The stomodeum was formed and transformed into the mouth in the early pentactula stage. After 10 d of development, the primary tentacles began to push out of the vestibule, which was almost at the very anterior end of the early pentactulae. By 13 d, all embryos had developed into well-defined pentactulae, having five buccal podia and an anal pore. At that time, the preoral lobe completely disappeared.

During development in culture, most mortalities occurred within the first 20 days up to metamorphosis (Fig. 2A). Fertilization was the first step in which a large percentage of oocytes (ca. 32%) that were not successfully fertilized died (Fig. 2B). During the following hatching, 59% of surviving embryos died; finally, during metamorphosis, ca. 50% died. Afterward, the remaining population of young sea cucumbers (ca. 15% of the original quantity of oocytes) did not decrease significantly and only a few individuals died occasionally, without obvious reasons (Fig. 2B).

Development under controlled environmental conditions and sperm concentrations

The monospermic fertilization success increased with sperm concentration, independent of the oocyte densities tested (Fig. 3). At the lowest concentration (0.1×10^4 spermatozoa/mL), <6% of the oocytes were fertilized whereas at 2.5×10^4 spermatozoa/mL the success rate reached ca. 82%. Evidence of polyspermy, leading to mortality, first appeared at 2.5×10^4 spermatozoa/mL in ca. 2% of the oocytes and this proportion increased progressively to reach a maximum (75%) with 20×10^4 spermatozoa/mL at all oocyte concentrations tested (Fig. 3). When fertilization rates, tested at different gamete concentrations, were combined as a function of spermatozoa:oocyte ratios, a maximal rate of monospermic fertilization was obtained for a spermatozoa:oocyte ratio ranging from $7 \times 10^4 : 1$ to $1 \times 10^6 : 1$.

Fig. 1. Photographic sequence illustrating the principal stages of early development of *Cucumaria frondosa* observed in the laboratory. For time and size evolution, refer to Table 1. (A) Fertilized eggs showing the completely elevated fertilization coat (arrow), (B) 4-cell stage, (C) embryo reaching the 32-cell stage by a clear asynchronous division between the blastomeres of the vegetal (*v*) and animal (*a*) poles, (D) persistent asynchrony of blastomere cleavage after 30 h of development, (E) blastocoele (*bc*) visible across the thin epithelium of this well-developed blastula following the compaction of blastomeres, (F) late gastrula after elongation (late gastrula) showing the blastopore (*b*), (G) vitellaria showing the vestibule (*vt*), the podial pit (*p*), and the residual blastopore (*b*), (H) newly settled pentactula with a pair of ambulacral podia (*ap*), showing the anal pore (*an*) and the five tentacles (*t*) beginning to ramify, and (I) 10 mm long young sea cucumber after 13 months of growth, showing the ambulacral podia (*ap*), the numerous ossicles (*o*), and the tentacles (*t*). (A) Scale bar = 250 μm (also applies to B, C, and F). (E) Scale bar = 520 μm (also applies to D). (G) Scale bar = 540 μm. (H) Scale bar = 490 μm. (I) Scale bar = 2500 μm.



We found that pH 8 increased fertilization success and developmental kinetics, reducing the time between each successive stage (Fig. 4). Below and above this pH level, the kinetics and the survival rate decreased. When pH was adjusted to 7.5, development stopped at the two-cell stage. No fertilization occurred between pH 6 and 7, and development at pH 9 was ca. 31% longer than at pH 8. The influence of pH became clearer as development progressed, with the strongest disparities observed in the time needed to attain the pentactula stage with differences up to 4 d, depending on pH conditions (7–9).

The best fertilization success and fastest development were at a salinity of 26 psu (Fig. 4). Development took 30% longer when salinity was adjusted to 34 psu, but no influence on mortality rates was recorded in the culture. No fertilization occurred at salinities ranging from 20 to 22 psu. Microscopic observations of the oocytes from all salinities tested indicated no cell shrinkage or lysis.

Development was totally prevented at 15°C or higher (Fig. 4). The best fertilization success and highest developmental kinetics were at 12°C and they progressively slowed to 0°C (Fig. 4). However, cold temperatures (0–10°C) did not increase mortality at each step of development.

Larval settlement

Embryos in the laboratory remained buoyant until the vitellaria stage (7 d after fertilization) as long as uniform ciliation persisted. Larvae at this stage had a very constant pattern of activity and swam actively (12.4 ± 4.3 cm/h). When the larva developed into pentactula with five buccal podia on the oral end of the body, and further, when two ambulacral podia developed, the cilia progressively disappeared (43–48 d after fertilization) (Fig. 1, Table 1). The proportion of inactive cilia and denuded sections of the embryo's surface increased progressively. During this transformation, achieved in 3–8 d, the

Table 1. The time and size evolution of embryos of *Cucumaria frondosa* up to young sea cucumbers, under natural environmental conditions reproduced in the laboratory.

Stage of development	Time	Size (mm)
Fertilized oocytes	0 min	0.90±0.2
Early stage of fertilization membrane elevation	2.0±0.1 min	1.10±0.3
Fertilization membrane completely elevated	5.0±0.2 min	1.40±0.3
First polar body	9.0±0.2 min	1.30±0.2
Second polar body	22.0±0.4 min	1.30±0.3
Vacuoles exocytosis	4.5±1.2 h	1.30±0.2
Beginning of first cleavage	5.5±1.3 h	1.30±0.2
2-cell	6.5±1.1 h	1.30±0.3
4-cell	8.5±1.3 h	1.35±0.4
8-cell	11.5±1.7 h	1.30±0.3
16-cell	14.0±1.4 h	1.35±0.3
32-cell	21.0±2.0 h	1.35±0.4
64-cell	24.0±2.1 h	1.40±0.5
128-cell	28.0±2.0 h	1.35±0.4
Young blastula (compaction)	35.0±2.4 h	1.30±0.5
Blastula	48.0±3.6 h	1.35±0.3
Spinning	59.0±5.5 h	1.40±0.4
Hatching	62.0±8.0 h	1.30±0.4
Young gastrula	72.0±8.5 h	1.30±0.3
Late gastrula	4.3±0.5 d	1.40±0.5
Elongation	5.5±0.5 d	1.60±0.5
Vitellaria (uniformly ciliated)	8.0±1.0 d	1.55±0.3
Young pentactula (5 tentacles)	11.0±1.5 d	1.30±0.3
Appearance of the anal pore	13.0±1.0 d	1.35±0.4
Late pentactula (with pair of ambulacral podia)	17.0±1.4 d	1.40±0.5
Settlement (cilia loss)	46.0±2.0 d	1.40±0.4
Young sea cucumber with 2 pairs of ambulacral podia	3.0±0.2 months	1.70±0.5
Young sea cucumber with 5 pairs of ambulacral podia	4.0±0.2 months	1.90±0.5

Note: The temperature recorded throughout the development varied from 0 to 13°C (Fig. 1). A new stage was considered attained when 50–60% of the embryos reached it.

embryos sank (up to 32 ± 10 cm/h) and regularly attached to the substrate for short periods of time (≤1 h). They came in contact with the substrate 12–15 times each day and did not disperse more than 10 mm from the bottom. For a short transition time, the larva remained able to float and regularly touch the substrate with its tentacles. At that time the reddish color characteristic of the embryo was also lost and replaced by a brownish translucent color.

For all experiments tested, the position of a substrate in the checkerboard design had no influence on larval abundance ($\chi^2_{0.05,25}$ $p \leq 0.01$). Consequently, boxes close to the periphery of the tank, in the central part, or close to the source of current were not favored by pentactula during settlement. For all experiments using living pentactula, 8–15% of the larvae died before the end of the observation, and no aggregative behavior was observed.

Influence of current and light

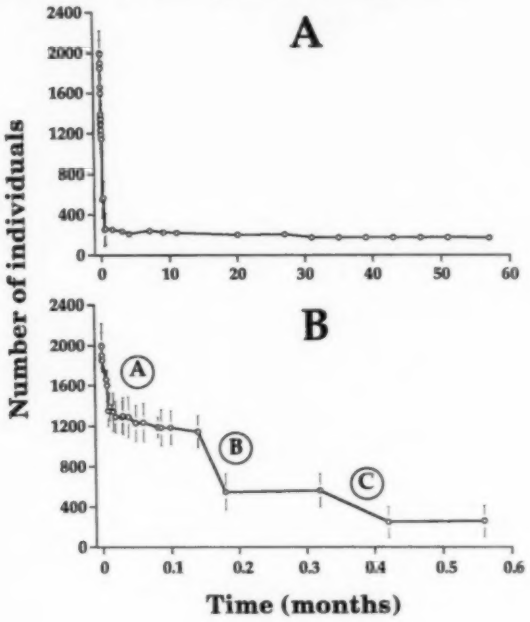
The settling individuals not only favored the sides and undersurfaces of gravel or rock, but were also very sensitive to illumination (Fig. 5). Under lighting, the percentage of definitive settlement was significantly higher ($\chi^2_{0.05,25}$ $p \leq 0.01$) on rocky substrate with crevices (between 38 and 46%) or with a smooth surface (20–22%) and on gravel (25–33%) than on all other substrates (≤11%) (Fig. 5). The effect of current on substrate selection was negligible. Settling of pentactula showed no statistical difference ($\chi^2_{0.05,25}$ $p = 0.731$) between turbulent and still water conditions (Fig. 5).

In ≥69% of pentactulae tested, definitive settlement (indicated by the clear attachment of the first two ambulacral podia on the substrate, Fig. 1H) occurred when the pentactula found a shady rock surface. The search for a shady substrate was accomplished through a rapid migration, initiated <48 h after the first contact with the substrate (Fig. 6). Moreover, this migration was completed by 52% of the pentactulae when they found a crevice that was ≤5 mm wide (Fig. 6). Final settlement of larvae coincided with a total loss of buoyancy. The settled sea cucumbers on other substrates (glass, mud, or sand) grew after settlement, but mortality increased by 37% and the growth rate diminished by 21% for the same period of observation (ca. 2 months) when compared with those settled on gravel or rock surfaces.

When the same settling experiment was done with dead larvae, in both current and still water conditions, a uniform distribution of the larvae on all substrates offered was observed (Figs. 5, 6). Therefore, most larvae settled on the upper surface of the rock and only a few were found on the undersurface (Fig. 5).

The exploration behavior before settlement lasted for 24–48 h in total darkness. The results confirmed a net

Fig. 2. (A) Incidence of mortality among *Cucumaria frondosa* embryos at each step of development. (B) Magnification of the 15 first days of the experiment. The circled letters correspond to fertilization (A), hatching (B), and metamorphosis (C).



preference of the pentactula for rocky substrates; the results of the experiment in darkness were no different from those of the experiment in the light, when each test substrate was compared ($\chi^2_{0.05,25}$ p = 0.074–0.243) (Fig. 5). The glass bottom, mud, and sand were largely ignored by the pentactulae (<10% of settlement); the gravel and rock surfaces were favored (90%). The pentactulae uniformly colonized all of the rock and gravel surfaces offered, showing no significant preference for either upper surfaces or undersurfaces in both still ($\chi^2_{0.05,25}$ p = 0.236) and turbulent water conditions ($\chi^2_{0.05,25}$ p = 0.073) (Fig. 5). However, a migration similar to that observed in the previous experiment was initiated with the return of light–shade conditions, provoking the newly settled larva to move to the shady areas. When the darkness was maintained for more than 12–15 h, a growing number of larvae did not progress to the postsettling migration, increasing from ca. 15% after 12 h to >50% after 25 h.

Influence of other factors

Larvae did not significantly prefer to settle on substrates either covered or not with ectodermic jelly from adult sea cucumbers ($\chi^2_{0.05,25}$ p = 0.127–0.643). When natural (untreated) substrates were tested, preference was not statistically different from that for heated substrates ($\chi^2_{0.05,25}$ p = 0.094–0.427) (Fig. 6).

Long-term growth and postsettling migration

Laboratory experiments

After settlement, the growth of the pentactula continued by further elaboration of the gut into a simple loop and proliferation of dermis calcareous plates (Table 1). First noticed around the anus, thin perforated calcareous plates soon covered the body. They appeared to be similar to the ones observed on adults: free of spines and surrounded by knobs. By this time, the tentacles began to ramify and numerous papillae developed on the ends. At this time (48 d after fertilization) the embryos actively accepted large amounts of phytoplanktonic cells, principally diatoms (*Skeletonema costatum*, *Chaetoceros debilis*, *Ceratium longipes*). They caught their food with their tentacles and transferred it to the mouth. The faeces were strings of pellets. Three months after fertilization, the second pair of ambulacral podia appeared; after 4 months, a total of five pairs were fully developed. The young were 10 mm in length with the same number of ambulacral podia after 13 months. Pumping movement of the anus was also observed. Continuously fed individuals attained a maximal length of 35 mm after 24 months of growth (Fig. 7). At this stage of development, they showed adult behavior.

The first 20 months of growth in the laboratory (Fig. 7) showed a pattern consisting of an initially slow absolute growth rate:

$$y = -0.45254 + 0.9169t, \qquad r^2 = 0.97$$

where t represents the time from fertilization (in months), and y represents the corresponding individual's length (in millimetres). During this time, the young first elongated until they reached a length:width ratio of 3:1 at 34 mm, after which their size continued to increase but their length:width ratio diminished (3:2).

Field experiments

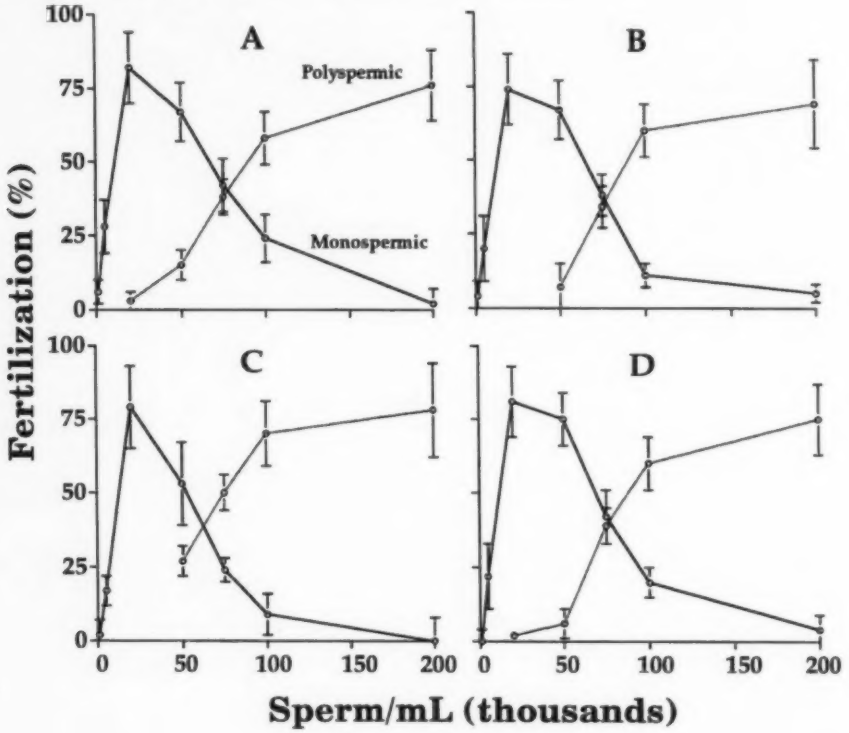
Growth rates of *Cucumaria frondosa* at different depths are shown in Fig. 8. The growth rate of young usually increased significantly and synchronously in both males and females with spring phytoplanktonic production and increasing temperature and was reduced during the colder and less productive winter months (Figs. 7, 8, 9) (Z test, Tessier's slope analysis, $p \leq 0.01$). The maximal size (107 mm) was attained in 40 months by the sea cucumbers at 20 m depth, which also showed the highest mean growth rate (ca. 2 mm/month) and spring and summer growth rate (5–6 mm/month). Their mean growth rate was 8 times higher than that of sea cucumbers found between 0 and 10 m (0.25 mm/month) (Fig. 8). Although still visible at 10 and 15 m, the spring–summer bursts were no longer detected for individuals at 0 and 5 m (Fig. 8).

When individuals at 20 m depth attained a size of 30–40 mm following a relatively slow growth rate (1.8 mm/month), a period of faster growth was initiated. The individuals increased their size with a maximal rate of 6 mm/month (Fig. 8). No difference was observed between male and female growth rates (to the maximal size obtained in the field) at any depths (Figs. 7, 8) and their common best fit at 20 m followed a general equation:

$$y = 32.009 + 1.7533t, \qquad r^2 = 0.98$$

where t represents the time from fertilization (in months) and y represents the corresponding individual's length (in millimetres). From the initial size until the end of the 40 months of growth, the average size of the young sea cucumbers had increased by ≈770% at 20 m depth, by 420% at 15 m, by 320%

Fig. 3. Effect of *Cucumaria frondosa* oocyte and sperm concentrations on fertilization rate. The percentages of monospermic and polyspermic fertilizations under increasing sperm and oocyte concentrations are given for (A) 100 oocytes, (B) 500 oocytes, (C) 1000 oocytes, and (D) 1500 oocytes. The error bars represent the confidence interval (95%).



at 10 m, by 110% at 5 m, and by 90% at 0 m. Of the 43 *Cucumaria frondosa* followed to sexual maturity, none reached the maximal size usually reached by this species in the field (ca. 350 mm). After 5 years, the animals attained only a maximal size of ca. 107 mm at 20 m depth. An extrapolation of the growth curve in Fig. 6 (in the field at 20 m depth), supposing a continuous growth rate, allowed us to estimate that a minimum of 10 years are necessary for an individual to attain the maximal size observed in the field under natural environmental conditions.

At 20 m depth, where the growth rate was maximal (Figs. 7, 8), *Cucumaria frondosa* usually initiated gonad synthesis in early spring of the first year of development. The onset of gametogenesis was related to size as well as age. The largest individuals observed were the first to initiate gametogenesis. The smallest from which a recognizable gonad sample was obtained was 38 mm in length (Fig. 8). At this size, the gonad was sexually undetermined with 10–15 very short (<8 mm long) gonadal tubules. It became possible to identify the sex only when the individuals were around 45–55 mm. The first oogonia or spermatogonia along the germinal epithelium were then visible. Sexual maturity was attained when individuals reached lengths of 80–102 mm during spring 1993, after 33 months of growth. The gonads were small and only a fraction of the oocytes (35–70%) and a few spermatozoa were fully developed. At that time, 35–50 gonadal tubules (ca. 20 mm long) were present, pink in males and brownish in females. Sexual maturity appeared more rapidly in deeper water, after ca. 3.8 years at 15 m and ca. 2.8 years at 20 m depth. Sea cucumbers never attained sexual maturity in any other shallower depths during the same experimental period.

Postsettling migration

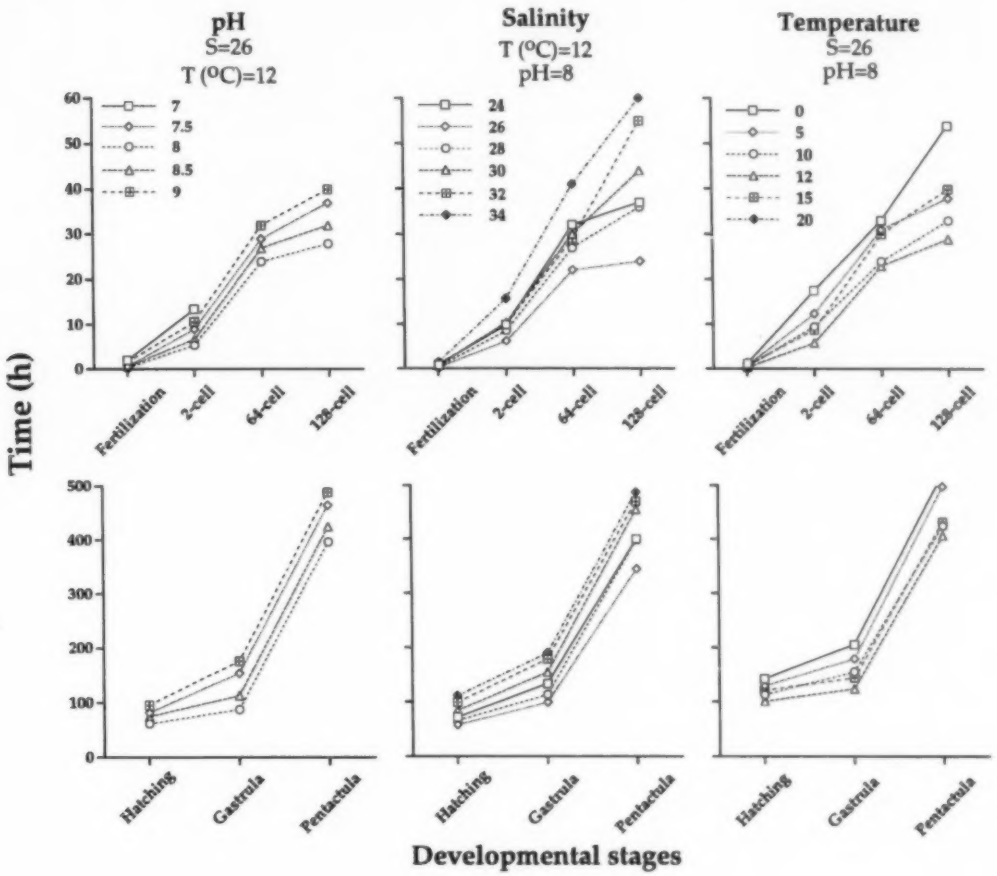
After reaching ca. 2.8 mm in length (4–5 months after the first contact with the substrate), the young (ca. 6 months old) progressively moved to the illuminated surface always on rocky surfaces, but stayed in protected areas like crevices. This movement occurred for all individuals ($n = 90$) over a 2-week period. Finally, they migrated (ca. 2.5 mm/h) out of the crevices and other protected areas of the substrate only when the minimal safe size of ca. 35 mm was attained (after ca. 2 years of growth). The sea cucumbers always made these movements using the ambulacral podia alone.

Seasonal spatial distribution in the field

Location of young sea cucumbers on the substrate

During early summer 1992, between 61 and 83% of the small individuals under 30 mm were found on the undersurfaces of rocks or gravel, especially confined in crevices or between rocks (Table 2). Young sea cucumbers occasionally occurred in shells of dead mollusks. Only 17–38% of small individuals

Fig. 4. Developmental kinetics of *Cucumaria frondosa* under various pH, salinity, and temperature conditions. The data are presented as the mean of three different experiments where the maximal standard deviation recorded was ca. 10%.



were found on the combined sides and top of the substrate. In contrast, 78–89% of the larger individuals over 30 mm were mainly found on those areas of the substrate (Table 2).

Size distribution with depth, sex, and season

Cucumaria frondosa were well represented from 0 to 60 m depth, mainly on hard substrates. The spatial distribution of immature and mature individuals was distinct. The smaller individuals were found mainly in the shallow water (<20 m) and the larger ones in deeper water (≥20 m) (Fig. 10). Moreover, the size structure also varied greatly with time from spring 1992 to fall 1993, especially for younger individuals (≤130 mm long) (Fig. 10).

Newly settled individuals (<30 mm in length) were found principally in the photic zone between 0 and 20 m depth and represented ca. 15% of the individuals present. Between 20 and 40 m, those same young sea cucumbers accounted for ca. 1% of the population. Below 40–60 m depth, in the aphotic zone (Babin et al. 1993), very young sea cucumbers were virtually absent (Fig. 10). The maximal density of settled pentactulae and young sea cucumbers was noted in the areas where the boulders, small rocks, and gravel were the most abundant substrates and also corresponded to the brightest areas studied.

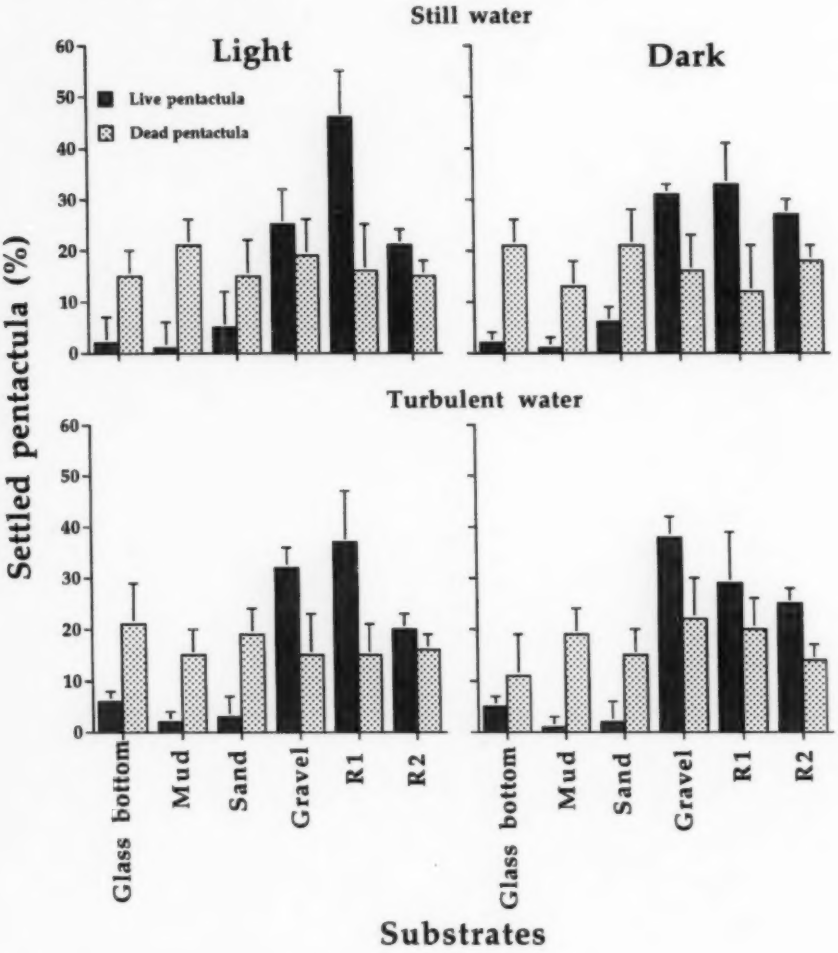
Table 2. Distribution of the different size-classes of young sea cucumbers (*Cucumaria frondosa*) on boulders in the field (n = 3400).

Location on boulder	Size-classes (mm)				
	0–10	10–20	20–30	30–40	40–50
Top	2	10	15	52	45
Side	15	11	23	37	33
Undersurface	83	79	61	11	22

Note: Percentages in each size-class sum to 100.

Individuals of all sizes under 130 mm in length were present between 0 and 20 m in spring and in summer 1992 (Fig. 10). However, in fall 1992 and early winter 1993 the largest of these individuals (ca. 60–130 mm) became significantly less abundant. Inversely, they were observed only in very low abundance in deeper areas (20–60 m depth) in spring

Fig. 5. Substrate selection by pentactula larvae (n = 200) of *Cucumaria frondosa* in still and turbulent water. The different substrates were tested in light and dark conditions and the results for dead and live larvae are presented. The error bars represent the confidence interval (95%) calculated with triplicate experiments in the checkerboard design tank. R1, rocks with crevices smaller than 5 mm; R2, rocks with smooth surface.



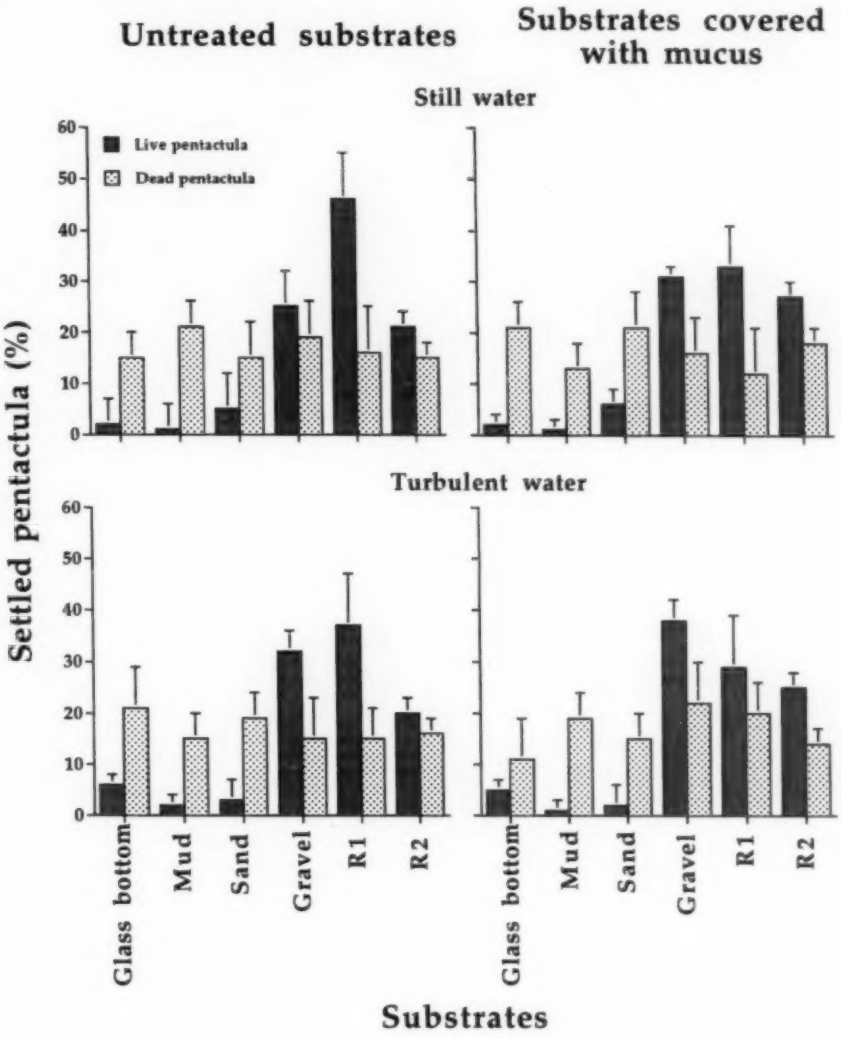
and in summer, and showed a sudden increase in abundance in fall 1992 and early winter 1993 (Fig. 10). During spring 1993 the individuals of 60–130 mm reappeared at 0–20 m. This size corresponds to the size at sexual maturity. The migration coincided with the first significant decrease in water temperature in fall and early winter, and with a concurrent decrease in food availability. Again, in fall 1993, the group of individuals between 60 and 130 mm almost disappeared from the shallower water and were found in deeper water (20–40 m).

Observations of sea cucumbers on muddy or sandy bottoms were rare during spring and summer. They became more frequent in fall and early winter when over 52 direct observations of moving sea cucumbers were made, compared with only 2 observations during spring and summer. Individuals were of various sizes but the vast majority were between 60 and 130 mm and their speed (recorded along graduated bars using SCUBA) varied from 2 to 7 cm/h. All individuals were sexually mature. In deeper water (20–60 m) the size of the individuals varied from 60 to 350 mm. Individuals smaller than 60 mm and newly settled ones were rarely found at those depths (Fig. 10).

Abundance of major predators

The abundances of the sea star *Solaster endeca* and of the green sea urchin *Strongylocentrotus droebachiensis* were evaluated every season at the usual depths. The maximal density of the sea star occurred at 20 m depth (ca. 0.1 individuals/m^2). Few were observed at depths shallower than 15 m. *Strongylocentrotus droebachiensis* were observed from the LWST to 15 m depth in high densities (between 125–375 individuals/m^2). Below this depth, the number of sea urchins decreased rapidly and virtually none were observed below

Fig. 6. Substrate selection by pentactula larvae (n = 200) of *Cucumaria frondosa* in turbulent water for the natural substrate (not cleaned) and substrate covered with the mucus of adult sea cucumbers. The different substrates were tested under light conditions and the results for dead and live larvae are presented. The error bars represent the confidence interval (95%) calculated with triplicate experiments in the checkerboard design tank. R1, rocks with crevices smaller than 5 mm; R2, rocks with smooth surface.



40 m depth. Direct observation of predation on the sea cucumbers remained scarce over the study period. Four cases involved the sea star *Solaster endeca* preying on individuals under 250 mm long. *Strongylocentrotus droebachiensis* was observed to prey on sea cucumbers under 25 mm on 15 occasions whereas no predation on bigger individuals was noted.

Discussion

The development of *Cucumaria frondosa* is slow and sensitive to various environmental conditions; mortality before settlement is high. The pentactulae show a strong photoreaction and substrate discrimination during the well-defined exploration and settlement periods. Subsequent migratory behaviors can be observed, showing that the settlement is only a part of the mechanism that explains the location of individuals on the substrate during their entire life (Fig. 11).

Spawning

Aside from spontaneous gamete release occurring after collection, artificially induced spawning in sea cucumbers has been rarely recorded (see review by Smiley et al. 1991). In the laboratory, female *Cucumaria frondosa* spawned when

Fig. 7. Growth of embryos and young *Cucumaria frondosa* under natural environmental conditions during the first 24 months in the laboratory (n = 30–40) and in the field at 20 m depth during 40 months (n = 80–90). The young were monitored from fertilization to ca. 35 mm in length in the laboratory, and from 35 to ca. 110 mm in the field. The error bars represent the confidence interval (95%).

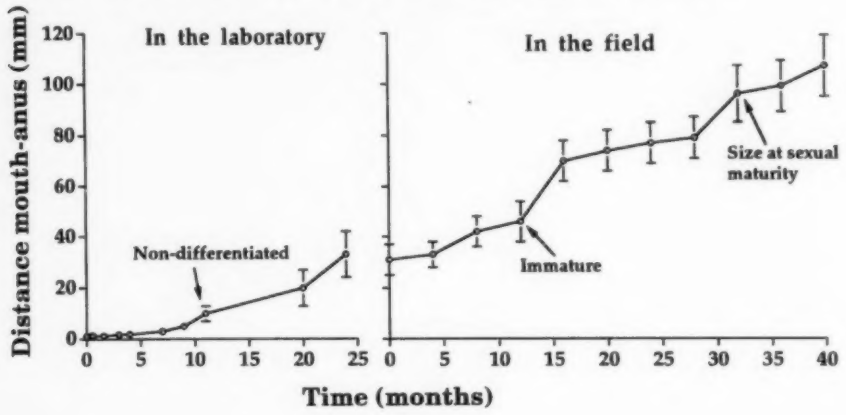
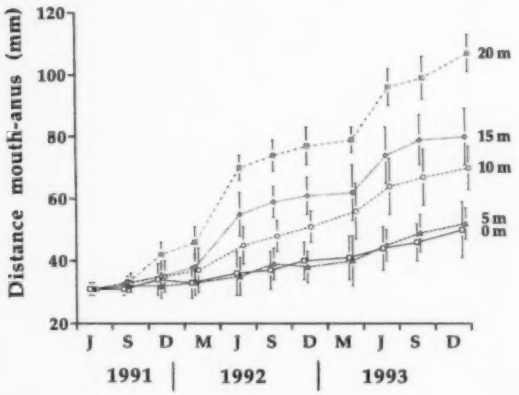


Fig. 8. Evolution of environmental factors for *Cucumaria frondosa* in the field at 20 m depth from June 1991 to December 1993 and in the laboratory from May 1992 to November 1993.



exposed to a drastic change in light regime combined with increasing temperature. This procedure stimulated spawning in up to 55% of individuals, and no other technique was successful. In other species of sea cucumber, such as *Psolus chitonoides* (McEuen and Chia 1991), a spawning was successfully artificially induced in males and females by either turning off the water circulation or warming the water. Chia and Buchanan (1969) triggered spawning in male and female *Cumaria elongata*, and Colwin (1948) in *Thyone briareus*, by raising the water temperature. Temperature thus plays a key role in the spawning of sea cucumbers, at least in species from temperate latitudes.

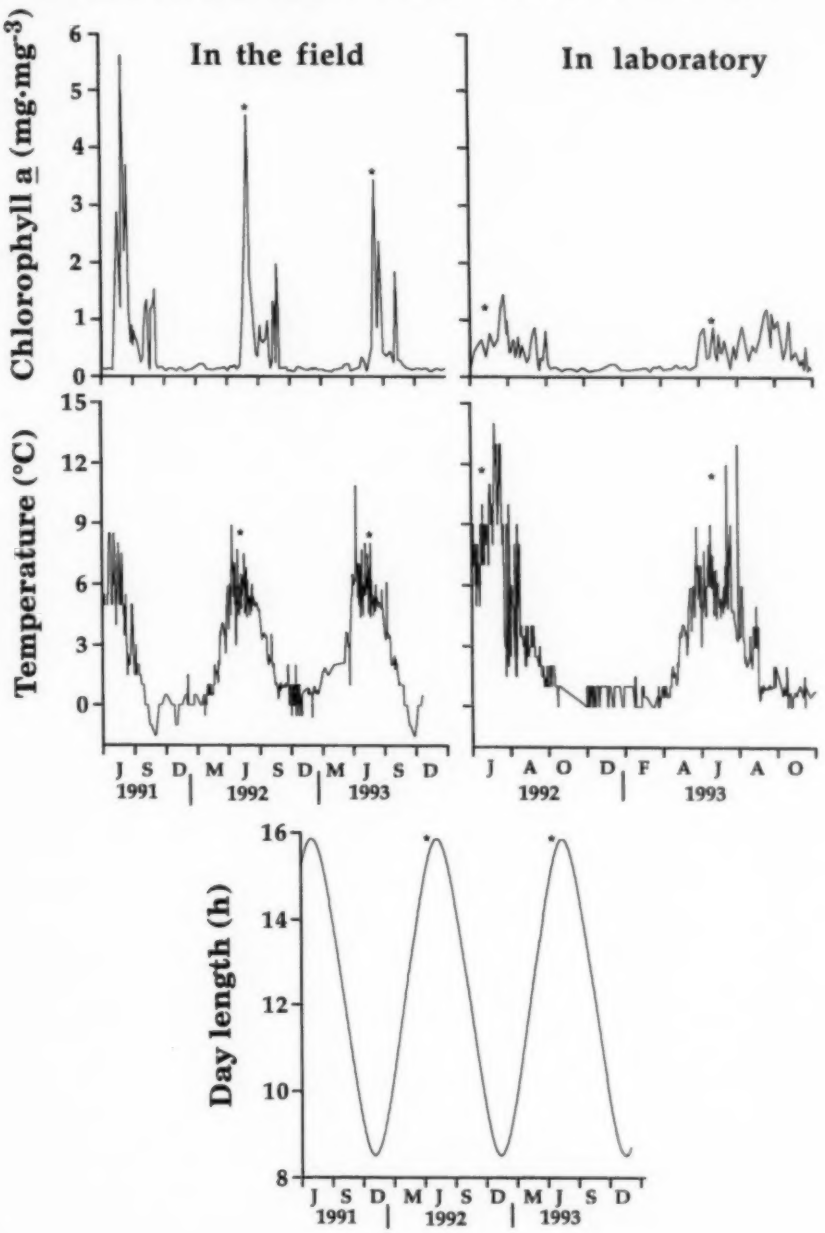
In the laboratory, *Cucumaria frondosa* spawned for ca. 3 h. The contraction of the body wall (from the anus to the mouth) lasted ca. 2 min and coincided with a continuous release of gametes. As adults possess around 70–80 mature gonadal tubules, the duration of a contraction seems to be related to the time needed to empty a single tubule. It appears that the peristaltic movements are involved in the release of gametes by helping to contract the tubules.

The spawning individuals (male or female) did not eat their own gametes either during or after their release. *Cucumaria frondosa* are very selective feeders and eat only a few species of phytoplankton among the many present in their habitat (J.-F. Hamel and A. Mercier, personal observations). It is therefore not surprising that the adults should have a good discriminating ability toward their own gametes. Two main mechanisms prevent the consumption of embryos: (*i*) both oocytes and embryos are positively buoyant, and (*ii*) adults do not deploy the fine ramifications of their tentacles during spawning. A possible chemical stimulus from the sperm and oocytes could also affect chemosensory cells on the tentacles, with varying specificity.

Development

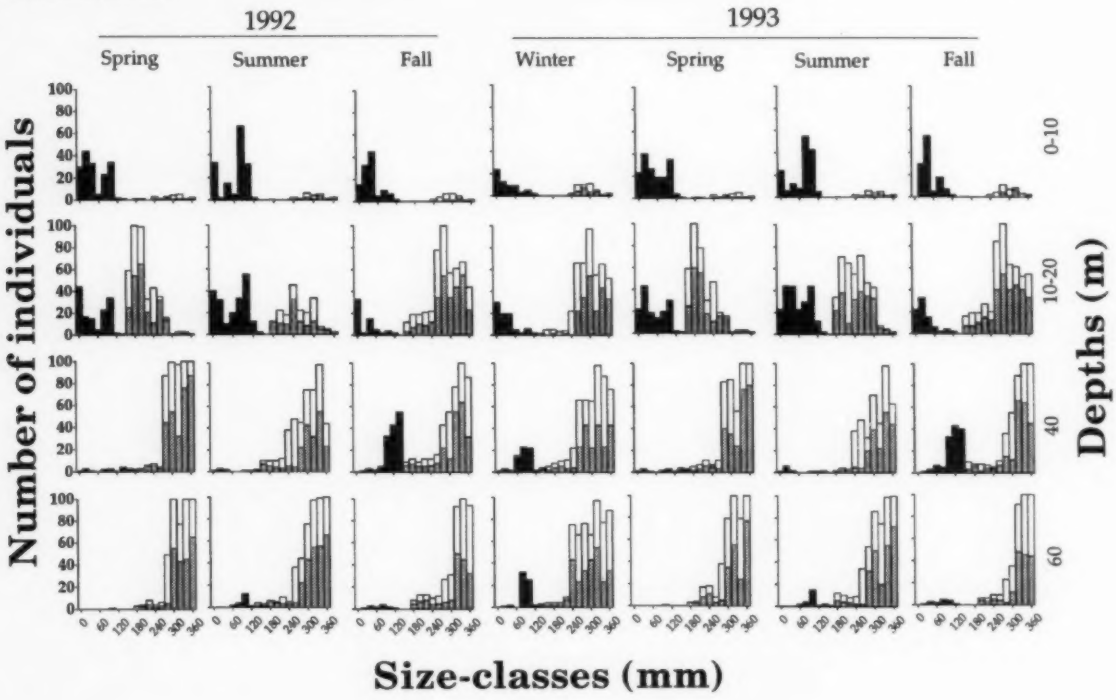
The development of *Cucumaria frondosa*, from the 32-cell to the 256-cell stage, showed a clear developmental asynchrony between the vegetal and animal pole, with the animal hemisphere dividing faster than the vegetal one. This was also observed by Arts (1910) and Runnström and Runnström (1919). Asynchronous cleavage is common in holothurian embryos, as observed by Inaba (1930) for *Paracaudina chilensis* and by Holland (1981) for *Stichopus tremulus*. We suggest that this asynchronous development may in part be due to the irregular distribution of reserves in the embryo, as inferred from the nonuniform density of the yolk inside the oocytes. Rustad (1938) suggested that poor culture conditions initiated such a phenomenon in *Stichopus tremulus*. However, in our experiments, asynchronous development occurred in every developing embryo and under all experimental conditions, therefore minimizing this possibility. Arts (1910) called the less divided cells of the vegetal pole a syncytium (a cell with more than one nucleus). Our observations indicate that these cells possess only a single nucleus and are not different, except in size, from the other cells of the embryo.

Fig. 9. Growth of *Cucumaria frondosa* at 0, 5, 10, 15, and 20 m depths at Les Escoumins, Lower St. Lawrence Estuary, from June 1991 to December 1993. The error bars represent the confidence interval (95%) calculated from the three cages at each depth (30 individuals/cage).


In the field
In laboratory
Chlorophyll a (mg·mg-3)
Temperature (°C)
Day length (h)
J S D M J S D M J S D
1991 1992 1993
J A O D F A J A O
1992 1993

Fertilization success in *Cucumaria frondosa* is dependent on sperm concentration, while fertilization and development of the embryos are sensitive to pH, salinity, and temperature. The concentrations of oocytes had no detectable effect on fertilization success or on the proportion of polyspermic embryos at a given concentration of spermatozoa. The very low density of oocytes tested in the laboratory, corresponding to the densities found in the field (Hamel and Mercier 1996), may explain these results. The sperm:oocyte ratio was very high. Compared with the few other species of echinoderms studied, the number of spermatozoa necessary to obtain optimal monospermic fertilization was low (ca. 2.5×10^4 spermatozoa/mL), and when the concentration was above $5–10 \times 10^4$ spermatozoa/mL, the proportion of polyspermic fertilization increased

Fig. 10. Size structure of immature (solid bars), male (hatched bars), and female (open bars) *Cucumaria frondosa* from spring 1992 to fall 1993 at different depths from 0 to 60 m. The size-classes between 80 and 120 mm in length correspond to individuals that have just reached maturity.



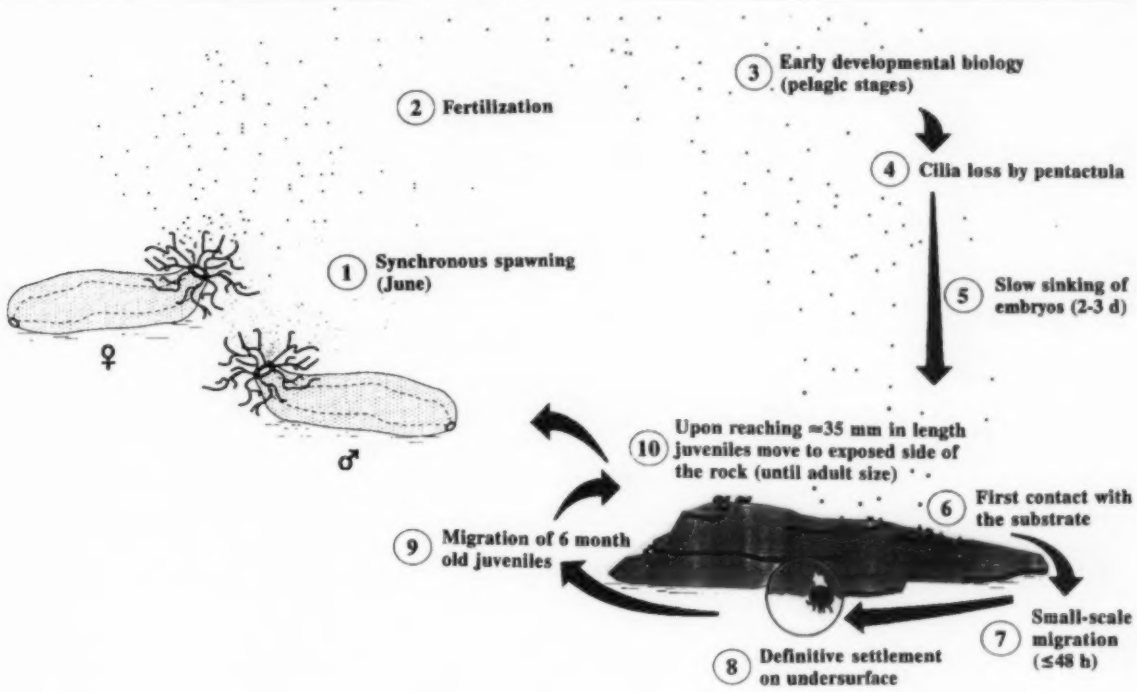
rapidly (Fig. 3). At least 10^6 spermatozoa/mL were required to obtain a high rate of fertilization in the sea urchin *Strongylocentrotus droebachiensis* (Pennington 1985). However, as mentioned by Levitan et al. (1992), the size of the oocytes seems to play a major role in the success of fertilization. In fact, the oocytes of *Cucumaria frondosa* have a volume ca. 3000 times greater than that of *Strongylocentrotus droebachiensis*, which may increase their chances of being fertilized.

Environmental factors seemed to play an important role during all stages of development. Jordan (1972) described the development of *Cucumaria frondosa* collected along the southern coast of Maine and indicated that 24 h were required to attain the morula stage, 3 d for the gastrula, and 9 d for the pentactula. In the St. Lawrence Estuary, the time necessary to attain these same stages was ca. 28 h, 4–5 d, and 9–12 d, respectively. This slightly longer development time may be due to the lower mean water temperature recorded in the St. Lawrence Estuary (annual mean 7°C) than on the northeastern coast of the United States (10°C, National Oceanic and Atmospheric Administration 1990). In the laboratory, all embryonic stages of *Cucumaria frondosa*, up to the pentactula, developed faster in warm water (12°C), with moderate salinity (26 psu) and a pH of 8. When those factors were adjusted lower or higher, development was significantly affected, either by showing increased time between each developmental stage, a total absence of development, or high mortality. Both survival and developmental duration in the various laboratory conditions tested suggest that this species prefers the estuarine conditions to the higher oceanic salinity and pH. In fact, the highest known densities of this species (considering just settled and adult individuals) can be found in the St. Lawrence Estuary (J.-F. Hamel and A. Mercier, personal observations) as well as in numerous estuaries along the Atlantic provinces of Canada (Ke et al. 1987). *Cucumaria frondosa* seems to be extremely well adapted to estuarine conditions, despite the lack of differentiated osmoregulatory and excretory organs (Drouin et al. 1985; Stickle and Diehl 1987).

Low temperatures tested in the laboratory did not kill *Cucumaria frondosa* embryos, but did result in a proportional decrease in the developmental kinetic. However, at 20°C the embryos did not develop, possibly because fertilization was prevented. As mentioned by Greenwood and Bennett (1981) and Masatoshi et al. (1984), extreme temperatures affect the fertilization capacity of sperm. While very cold temperatures around 0°C are common in the St. Lawrence Estuary, those over 12°C are quite rare.

The trials at various salinities and pH values also suggest that these factors affect fertilization success, with a very narrow range between optimal and lethal conditions. In fact, although they were viable at pH 7.5, embryos died at the two-cell stage at pH 7 and they developed normally at higher pH values with a maximum of effectiveness around pH 8. Clear signs of developmental changes were observed at the various salinities, but both survival and developmental rates suggest that this species is somewhat euryhaline. Moreover,

Fig. 11. Schematic illustration of the postsettling migration of the pentactula and young *Cucumaria frondosa* in the water column and on the bottom, during the first 6 months of growth observed in the laboratory.


1 Synchronous spawning (June)
2 Fertilization
3 Early developmental biology (pelagic stages)
4 Cilia loss by pentactula
5 Slow sinking of embryos (2-3 d)
6 First contact with the substrate
7 Small-scale migration (≤48 h)
8 Definitive settlement on undersurface
9 Migration of 6 month old juveniles
10 Upon reaching ≈35 mm in length juveniles move to exposed side of the rock (until adult size)
♀
♂

salinity affected the developmental duration, but in the range tested it did not affect the proportion of embryos that survived. The best results were at 26 psu.

Both temperature and salinity have been observed to cause fertilization alteration and developmental variability in many echinoderm species (Cameron et al. 1985; Pechenik 1987; Stickle and Diehl 1987; Roller and Stickle 1993) along with direct effects on the larva (Binyon 1972; Watts et al. 1982; Drouin et al. 1985; Roller and Stickle 1985). In addition, the effect of pH on fertilization success and development was described for the sea urchin *Strongylocentrotus purpuratus* (Dubé et al. 1985). Although the high temperatures and low salinities tested are not found in the site where the animals were collected, they may define the southern limit of the presence of *Cucumaria frondosa* along the east coast of North America. Those factors were also suggested to limit the distribution of other echinoderms (Thorson 1946; Fenchel 1965).

The overall conditions that reduce the delay between the stages of development are those found in the surface waters of the St. Lawrence Estuary. The optimal temperature for development is also that recorded along the coast of Maine. In fact, Therriault and Levasseur (1985) and Chassé (1994) indicated that the temperature during June and July around the study site reaches ca. 8–12°C close to the surface and is maintained near 2–5°C just a few metres below. When released in the water column in mid-June, the very buoyant oocytes were fertilized and their development mainly occurred near the surface. The embryonic stages subsequently showed an increasing sensitivity to environmental conditions. This pattern agrees with the idea that the embryos progressively sank from the very unstable surface water to deeper and more stable water, where they developed into pentactula.

The development monitored under the best environmental conditions showed that a proportion of the embryos died both before and upon reaching the settlement stage. In the laboratory, the vast majority of eggs and embryos died during the first month of growth. At least 45% of the eggs were lost before or during fertilization, suggesting that a proportion of the oocytes were not viable or that some were fertilized by more than one spermatozoan. A second high level of mortality was encountered during hatching. Some embryos stopped their development after showing great problems in extruding from the fertilization membrane. Finally, around 10% died during metamorphosis. Some of these seemed to have an incomplete digestive tract or abnormal external morphology, but the cause of death remained uncertain.

Settlement and migration

The settlement of the pentactula was divided into two distinctive steps: a primary search for the ideal substrate occurred as the larva began to lose buoyancy, followed by settlement itself. The growing embryo undertook a series of migrations, first toward protected and shaded areas, then toward lighted but still sheltered sites, and finally, further in the growth, toward the exposed substrate where food is abundant. Later, at sexual maturity, the sea cucumbers seemed to move again to relocate in deeper waters (Fig. 11).

Presettlement behavior

Because the loss of cilia and vitelline reserves was gradual, the pentactulae slowly lost buoyancy and explored the substrate, as their few remaining cilia and most probably the prevailing water flow helped them move about. McEuen and Chia (1991) proposed that because the larvae of the sea cucumber *Psolus chitonoides* were still positively buoyant at settlement, the descent through the water column was made possible by active swimming using the ciliary rings. They also showed that the cilia appeared just before the onset of vertical swimming behavior. This largely differs from the observations obtained from *Cucumaria frondosa* in which the cilia appeared much sooner, before hatching. The crawling of larvae over considerable distances prior to settlement was also observed by Young and Chia (1982) for *Psolus chitonoides*. The use of fixing organs, such as the microvilli and globules described by McEuen (1986) and McEuen and Chia (1991), may also be present in *Cucumaria frondosa* as Fankboner (1978), Smith (1983), and McKenzie (1985, 1987) indicated that they were a common feature in dendrochirotid holothurians. The precise mechanisms by which larvae explore and crawl about surfaces are not well known, but the fact that the pentactulae of *Cucumaria frondosa* either attach temporarily or delay their settlement suggests that they do not settle randomly on the substrate. In fact, during the period of exploration, the pentactulae were photopositive and mainly searched for a site inside the photic zone. Under darkness, in the laboratory, the period of exploration was prolonged over 15 h, until the light was turned on, indicating that the larvae were waiting to settle under lighted conditions.

Settlement

Settlement is one of the most critical phases in the life history of marine invertebrates. In echinoderms, the mechanisms of microhabitat selection during settlement are poorly known (see reviews by Chia and Walker 1991; Smiley et al. 1991; Pearse and Cameron 1991; Hendler 1991).

As soon as the pentactulae of *Cucumaria frondosa* attached their pair of ambulacral podia on the substrate, we considered that they had chosen a site for settlement. Given a choice between various types of substrate, the pentactulae generally preferred cryptic microhabitats, especially rock undersurfaces. They were found in significantly lesser numbers on sandy and muddy surfaces. These laboratory observations are largely consistent with the field studies during which no young sea cucumbers were noted on sand, silt, or algae, contrary to the statements of Jordan (1972) for the pentactula of *Cucumaria frondosa* along the coast of Maine. We suggest that the results described by Jordan (1972) were not representative of the final settlement but rather coincided with the exploration period occurring previously. Sloan (1979) noted that *Polycheira rufescens* was strongly associated with the presence of gravel and sand substrate under boulders. However, Cameron and Schroeter (1980) and Barker and Nichols (1983) indicated a lack of specific substrate selection for the sea urchins *Strongylocentrotus purpuratus* and *Strongylocentrotus franciscanus* and for the sea star *Asterias rubens*, respectively.

Our data reveal no major effect of moderate current conditions, corresponding to those found in the St. Lawrence Estuary. However, the presence of predators can apparently influence settlement, or survival after settlement. For instance, green sea urchins (*Strongylocentrotus droebachiensis*) were found to reach high densities in the sites where *Cucumaria frondosa* were observed. They may have an influence on the spatial distribution and abundance of young sea cucumbers because they eat only individuals under 30 mm long. Nonetheless, laboratory experiments in the absence of predators showed a comparable depletion of small sea cucumbers on the upper surfaces of the substrate. The pentactulae actively move to the undersurfaces. This observation suggests a possible evolution of the larval settlement to minimize predation rather than a depletion of sea cucumbers on the rock surfaces by predatory pressures. Interestingly, the settlement occurred at depths where the potential predators reach their maximal densities, but also where planktonic food is abundant and the temperature higher, which may favor the growth rate of individuals. No newly settled sea cucumber was found in close proximity to an adult in the field, nor did the young favor mucus-covered substrate in the laboratory. Those results differ from those of Young and Chia (1982) that indicated a gregarious settlement of *Psolus chitonoides* pentactulae near or on adults or other small individuals already settled.

Larval orientation to light and substrate are independent reactions. Largely photopositive during exploration and settlement, the pentactulae of *Cucumaria frondosa* rapidly changed their first site of settlement and moved to shady surfaces. This suggests a drastic modification of the reaction to light, from a positive to a negative phototactic behavior. Young and Chia (1982) also observed a strong photorepulsion of the larvae of *Psolus chitonoides* after settlement on the substrate. They also indicated that pelagic juveniles of *Psolus chitonoides* were negatively phototactic. McEuen (1986) reported that some species of holothurians are neutral to light whereas others are either positively or negatively phototactic. Although uniformly ciliated, the pentactula of *Cucumaria frondosa* showed a clear phototactic response as also described by Runnström and Runnström (1919), despite the suggestion made by McEuen (1986) that uniformly ciliated larvae do not display any photoresponse. In the field, most newly settled pentactulae of *Cucumaria frondosa* were found in the shallow and lighted water depths (≤20 m), and only very few were noted below.

Further migration

Cucumaria frondosa undertook an extensive two-stage migration ca. 4.5 months after the settlement. First, they progressively moved short distances from the undersurfaces toward sheltered and lighted areas. We suggest that this movement was related to the spatial restriction of the rock undersurfaces and by food availability that was not sufficient for growing animals. However, it is difficult to explain the migration of all individuals during the same month. At that time, the sea cucumbers measured ca. 11 mm and were still vulnerable to the grazing activities of the sea urchin *Strongylocentrotus droebachiensis*, as indicated by numerous observations in the laboratory and in the field. Remaining in crevices while coming out from under the rocks to where food is abundant appears to be a good compromise.

Second, when the individuals reached ca. 35 mm in length (19 months later), the young sea cucumbers exposed themselves to potential predators. Field observations indicated that sea urchins did not prey on sea cucumbers of this size, suggesting that this movement occurred when the individuals reached

a safe size. As for the predator *Solaster endeca*, its densities were so low that its effect was considered minimal.

Seasonal variation of spatial distribution with depth

Cucumaria frondosa have a well-marked size-related distribution according to depth, with the smallest individuals in the shallower part of the site and the largest ones in the deeper water. This distribution may reflect the seasonal migration of *Cucumaria frondosa*. In fact, the small number of large individuals in shallow waters, the significant preference of the pentactulae for settlement in shallow water, and the visible displacement of medium-sized sea cucumbers to the deeper water during autumn strongly support this possibility. Jordan (1972) indicated that the population of *Cucumaria frondosa* at Lamoine Beach (Maine) is composed of smaller animals in inshore water year round, with older animals moving offshore in winter and returning in the spring, producing a decrease in the number of individuals and in the total biomass inshore during the winter. Similar cases of distribution were described by Bulteel et al. (1992) for *Holothuria tubulosa*, by Choe (1963) for *Stichopus japonicus*, and by Yingst (1974) for *Parastichopus parvimensis*.

The sudden movement of *Cucumaria frondosa* between 60 and 100 mm (size at sexual maturity) from shallow to deep water during autumn is striking. The migration of *Cucumaria frondosa* occurred in early autumn when the temperature decreased rapidly. Choe (1963) indicated that the temperature gradient could affect the bathymetric location in *Stichopus japonicus*, as small individuals always occur less deeply than large ones. Also, Choe (1963) for *Stichopus japonicus* and Yingst (1974) for *Parastichopus parvimensis* indicated that the deeper location of large individuals might be related to food availability. However, this does not seem to be the case for *Cucumaria frondosa* because the density of phytoplanktonic cells is uniform in the first 100 m of the water column all year in the St. Lawrence Estuary (Therriault and Levasseur 1985, 1986). Drouin et al. (1985) showed that large green sea urchins (*Strongylocentrotus droebachiensis*) migrated to greater depths where salinities are more stable but again this fails to explain why the migration was seen in *Cucumaria frondosa* as no drastic change in the salinity occurs in autumn. The salinity of shallow water in the study site does fluctuate but almost exclusively during spring (at depths >20 m) (Chassé 1994), indicating that the salinity gradient does not seem to play an important role in the fall migration of *Cucumaria frondosa*. Himmelman et al. (1984) found that larger *Strongylocentrotus droebachiensis* were more sensitive to salinity fluctuation than smaller ones. This may explain in part why the shallow-water recruitment of *Cucumaria frondosa* did not seem to be affected by the highly fluctuating salinity while the larger animals were not found in great numbers there. The light intensity varied greatly with depth in the study site and may be a major factor limiting the migration or initiating this behavior. Bulteel et al. (1992) observed that the predatory pressure on *Holothuria tubulosa* was negligible. It also seems to be the case in *Cucumaria frondosa*, as the predators of large sea cucumbers (>100 mm) are quite rare. Finally, the migration may be due to density-dependent factors that may decrease the intraspecific competition, as the abundance of *Cucumaria frondosa* is very high in the study site. It seems that there are many reasons why this migration occurred in *Cucumaria frondosa* whereas the reason why it occurred when individuals reached the size of sexual maturity remains unknown.

Growth rate in the laboratory and in the field

Most estimations of the growth rate of marine invertebrates were made by back-extrapolating measures on large individuals (Ebert 1978; Gage and Tyler 1985; Zann et al. 1987; Conand 1988; McClintock et al. 1993). This work is one of the rare studies that continuously monitored the growth of the animals, from fertilization until at least the size at sexual maturity over years, in the laboratory and in the field.

The growth pattern of *Cucumaria frondosa* was largely influenced by the environmental conditions, such as the temperature, the food supply, and the degree of disturbance of the environment. In the laboratory during the first year, the growth rate was slow and individuals reached only ca. 10 mm in length as the gonad began to develop (Fig. 7). The individuals reached ca. 35 mm in length after 24 months. In the field, individuals grew more rapidly after that size and even more so in the cages from deeper water than from shallow water. Although uniform in shallow water, growth showed a seasonal pattern at deeper depths, where it increased significantly during the spring and summer periods. Similar seasonal variations in growth rate were observed by Cameron and Fankboner (1989) for *Parastichopus californicus*, by Rutherford (1973) for *Cucumaria pseudocurata*, by Guillou and Michel (1994) for the sea urchin *Sphaerechinus granularis*, and by Barker and Nichols (1983) for the sea star *Asterias rubens*. The poor availability of phytoplanktonic food and the low temperature in winter may explain the slow growth rates at that time. Conversely, the warmer and more productive months of spring and summer coincide with the periods of faster growth. The more stable environment (e.g., salinity, temperature, and turbulence) observed in deeper water in the St. Lawrence Estuary may also explain the higher growth rate in *Cucumaria frondosa* at those depths, as observed by Bulteel et al. (1992) for *Holothuria tubulosa* in the Mediterranean Sea.

Despite a relatively low annual recruitment and a slow growth rate, taking ca. 3 years to reach the size at sexual maturity and ca. 5.5 years to reach 120 mm, the sea cucumber *Cucumaria frondosa* seems well adapted to the St. Lawrence Estuary, where it remains very abundant.

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References

Arts, L. 1910. Ueber die ersten Entwicklungsstadien von *Cucumaria frondosa* unter Berucksichtigung einiger anormaler Verhaltnisse. Bergens Museums Aarbok No. 13. Bergen, Norway.

Babin, M., Therriault, J.-C., Legendre, L., and Condal, A. 1993. Variations in the specific coefficient for natural phytoplankton assemblages: impact on estimates of primary production. Limnol. Oceanog. **38**: 154–177.

Bachelet, G., and Butman, C.A. 1991. Les canaux expérimentaux de la Woods Hole Oceanographic Institution, USA et leur apport à la connaissance des processus d'établissement des larves d'invertébrés benthiques. Programme national sur le déterminisme du recrutement (PNDR), Informations, Centre national de la recherce scientifique, Banyuls-sur-Mer, France. pp. 16–20.

Barker, M.F. 1977. Observations on the settlement of the brachiolaria larvae of *Stichaster australis* (Verrill) and *Coscinasterias calamaria* (Gray) (Echinodermata: Asteroidea) in the laboratory and on the shore. J. Exp. Mar. Biol. Ecol. **30**: 95–108.

Barker, M.F., and Nichols, D. 1983. Reproduction, recruitment and juvenile ecology of the starfish, *Asterias rubens* and *Marthasterias glacialis*. J. Mar. Biol. Assoc. U.K. **63**: 745–765.

Binyon, J. 1972. Physiology of echinoderms. Pergamon Press, Oxford.

Birkeland, C.E., Chia, F.S., and Strathmann, R.R. 1971. Development, substratum selection, delay of metamorphosis and growth in the seastar *Mediaster aequalis* Stimpson. Biol. Bull. (Woods Hole), **141**: 99–108.

Buchanan, J.B. 1967. Dispersion and demography of some infaunal echinoderm populations. Symp. Zool. Soc. Lond. **20**: 1–11.

Bulteel, P., Jangoux, M., and Coulon, P. 1992. Biometry, bathymetric distribution, and reproductive cycle of the holothuroid *Holothuria tubulosa* (Echinodermata) from Mediterranean seagrass beds. Mar. Ecol. (Berlin), **13**: 53–62.

Cameron, J.L., and Fankboner, P.V. 1989. Reproductive biology of the commercial sea cucumber *Parastichopus californicus* (Stimpson) (Echinodermata: Holothuroidea). II. Observations on the ecology of development, recruitment, and juvenile life stage. J. Exp. Mar. Biol. Ecol. **127**: 43–67.

Cameron, R.A., and Schroeter, S.C. 1980. Sea urchin recruitment: effect of substrate selection on juvenile distribution. Mar. Ecol. Prog. Ser. **2**: 243–247.

Cameron, R.A., Boidron-Metairon, I., and Monterrosa, O. 1985. Does the embryonic response to temperature and salinity by four species of Caribbean sea urchins parallel to reproductive synchrony? *In* Proceedings of the 5th Coral Reef Congress, Moorea, French Polynesia, 27 May – 1 June 1985. Vol. 5. *Edited by* C. Gabrié. Antenne Museum-EPHE. pp. 273–278.

Chassé, J. 1994. Modélisation numérique tridimentionnelle dans l'estuaire du Saint-Laurent. Ph.D. thesis, Université du Québec, Rimouski, Que.

Chia, F.-S., and Buchanan, J.B. 1969. Larval development of *Cucumaria elongata* (Echinodermata: Holothuroidea). J. Mar. Biol. Assoc. U.K. **49**: 151–159.

Chia, F.-S., and Walker, C.W. 1991. Echinodermata: Asteroidea. *In* Reproduction of marine invertebrates: Echinodermata and Lophophorates. *Edited by* A.C. Giese, J.S. Pearse, and V.B. Pearse. Boxwood Press, Pacific Grove, Calif. pp. 301–353.

Choe, S. 1963. Biology of the Japanese common sea cucumber *Stichopus japonicus*, Selenka. Pusan National University, Pusan, South Korea.

Colwin, L.H. 1948. Note on the spawning of the holothurian, *Thyone briareus* (Lesueur). Biol. Bull. (Woods Hole), **95**: 296–306.

Conand, C. 1988. Comparison between estimations of growth and mortality of two stichopodid holothurians: *Thelenota ananas* and *Stichopus chloronotus* (Echinodermata: Holothuroidea). *In* Proceedings of the 6th International Coral Reef Symposium, 8–12 August, 1988, Townsville, Australia. Vol. 2. *Edited by* J.H. Choat. pp. 661–665.

Dolmatov, I.Y., and Yushin, V.V. 1993. Larval development of *Eupentacta fraudatrix* (Holothuroidea, Dendrochirotida). Asian Mar. Biol. **10**: 123–132.

Drouin, G., Himmelman, J.H., and Béland, P. 1985. Impact of tidal salinity fluctuations on echinoderms and mollusc populations. Can. J. Zool. **63**: 1377–1387.

Dubé, F., Schmidt, T., Johnson, C.H., and Epel, D. 1985. The hierarchy of requirements for an elevated intracellular pH during early development of sea urchin embryos. Cell, **40**: 657–666.

Ebert, T.A. 1978. Growth and size of the tropical sea cucumber *Holothuria* (*Halodeima*) *atra* Jager at Enewetak Atoll, Marshall Islands. Pac. Sci. **32**: 183–191.

Fankboner, P.V. 1978. Suspension-feeding mechanisms of the armoured sea cucumber *Psolus chitonoides*. J. Exp. Mar. Biol. Ecol. **31**: 11–25.

Fenchel, T. 1965. Feeding biology of the sea star *Luidia sarsi* Duben and Koren. Ophelia, **2**: 223–236.

Gage, J.D., and Tyler, P.A. 1985. Growth and recruitment of the deep-sea urchin *Echinus affinis*. Mar. Biol. (Berlin), **90**: 41–53.

Gosner, K.L. 1979. A field guide to the Atlantic seashore. Houghton Miffin Company, Boston, Mass.

Greenwood, P.J., and Bennett, T. 1981. Some effects of temperature–salinity combinations on the early development of the sea urchin *Parechinus angulosus* (Leske). Fertilization. J. Exp. Mar. Biol. Ecol. **51**: 119–131.

Guillou, M., and Michel, C. 1994. The influence of environmental factors on the growth of *Spaerechinus granularis* (Lamarck) (Echinodermata: Echinoidea). J. Exp. Mar. Biol. Ecol. **178**: 97–111.

Hamel, J.-F., and Mercier, A. 1995. Spawning of the sea cucumber *Cucumaria frondosa* in the St. Lawrence Estuary, eastern Canada. SPC Beche-de-mer Information Bulletin No. 7. South Pacific Commission, P.O. Box D5, Noumea Cédex, New Caledonia. pp. 12–18.

Hamel, J.-F., and Mercier, A. 1996. Gamete dispersion and fertilization success of the sea cucumber *Cucumaria frondosa*. SPC Beche-de-mer Information Bulletin No. 8. South Pacific Commission, P.O. Box D5, Noumea Cédex, New Caledonia. In press.

Hamel, J.-F., Himmelman, J.H., and Dufresne, L. 1993. Gametogenesis and spawning of the sea cucumber *Psolus fabricii*. Biol. Bull. (Woods Hole), **184**: 125–142.

Harrold, C., Lisin, S., Light, K.H., and Tudor, S. 1991. Isolating settlement from recruitment of sea urchins. J. Exp. Mar. Biol. Ecol. **147**: 81–94.

Hendler, G. 1991. Echinodermata: Ophiuroidea. *In* Reproduction of marine invertebrates: Echinodermata and Lophophorates. *Edited by* A.C. Giese, J.S. Pearse, and V.B. Pearse. Boxwood Press, Pacific Grove, Calif. pp. 355–511.

Himmelman, J.H., Guderley, H., Vigneault, G., and Drouin, G. 1984. Response of the sea urchin, *Strongylocentrotus droebachiensis*, to reduced salinities: importance of size, acclimation, and interpopulation differences. Can. J. Zool. **62**: 1015–1021.

Holland, N.D. 1981. Electron microscopic study of development in a sea cucumber, *Stichopus tremulus* (Holothuroidea), from unfertilized egg through hatched blastula. Acta Zool. (Stockholm), **62**: 89–111.

Inaba, D. 1930. Notes on the development of a holothurian, *Caudina chilensis* (J. Muller). Sci. Rep. Tohoku Univ. Fourth Ser. (Biol.), **5**: 215–248.

Jordan, A.J. 1972. On the ecology and behavior of *Cucumaria frondosa* (Echinodermata: Holothuroidea) at Lamoine Beach, Maine. Ph.D. dissertation, University of Maine, Orono, Maine.

Ke, P.J., Smith-Lall, B., Hirtle, R.W., and Kramer, D.E. 1987. Technical studies on resource utilization of Atlantic sea cucumber (*Cucumaria frondosa*). Can. Inst. Food. Sci. Technol. J. **20**: 4–8.

Keesing, J.K., Cartwright, C.M., and Hall, K.C. 1993. Measuring settlement intensity of echinoderms on coral reefs. Mar. Biol. (Berlin), **117**: 399–407.

Lambert, R. 1991. Recrutement d'espèces benthiques à larves pélagiques en régime mégatidal. Cas de *Pectinaria koreni* (Malmgren), Annelide Polychete. Ph.D. dissertation, Université de Rennes I, Rennes, France.

Levitan, D.R., Sewell, M.A., and Chia, F.-S. 1992. How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. Ecology, **73**: 248–254.

Lucas, J.S. 1984. Growth, maturation and effects of the diet in *Acanthaster planci* (L.) (Asteroidea) and hybrids reared in the laboratory. J. Exp. Mar. Biol. Ecol. **79**: 129–147.

Masatoshi, M., Hino, A., and Yasumasu, I. 1984. Effect of temperature on interaction between eggs and spermatozoa of sea urchin. Biol. Bull. (Woods Hole), **166**: 68–77.

McClintock, J.B., Hopkins, T., Marion, K., Watts, S., and Schinner, G. 1993. Population structure, growth and reproductive biology of the gorgonocephalid brittlestar *Asteroporpa annulata*. Bull. Mar. Sci. **52**: 925–936.

McEuen, F.S. 1986. The reproductive biology and development of twelve species of holothuroids from the San Juan Islands Washington. Ph.D. dissertation, University of Alberta, Edmonton, Alta.

McEuen, F.S. 1987. Phylum Echinodermata, class Holothuroidea. *In* Reproduction and development of marine invertebrates of the northern Pacific coast, data and methods for the study of eggs, embryos, and larvae. *Edited by* M.F. Strathmann. University of Washington Press, Seattle, Wash. pp. 556–596.

McEuen, F.S., and Chia, F.-S. 1985. Larval development of a molpadiid holothuroid, *Molpadia intermedia* (Ludwig, 1984) (Echinodermata). Can. J. Zool. **65**: 2553–2559.

McEuen, F.S., and Chia, F.-S. 1991. Development and metamorphosis of two psolid sea cucumbers, *Psolus chitonoides* and *Psolidium bullatum*, with a review of reproductive patterns in the family Psolidae (Holothuroidea: Echinodermata). Mar. Biol. (Berlin), **109**: 267–279.

McKenzie, J.D. 1985. The tentacular ultrastructure of dendrochirote holothurians. *In* A comparative SEM study: Echinodermata. *Edited by* B.F. Keen and B.D.S. O'Connor. A.A. Balkema, Rotterdam, the Netherlands. pp. 445–450.

McKenzie, J.D. 1987. The ultrastructure of the tentacles of eleven species of dendrochirote holothurians studies with special reference to the surface coats and papillae. Cell Tissue Res. **248**: 187–199.

National Oceanic and Atmospheric Administration. 1990. Climatological atlas of the world ocean. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, U.S. Government Printing Office, Rockville, Md.

Pearse, J.S., and Cameron, R.A. 1991. Echinodermata: Echinoidea. *In* Reproduction of marine invertebrates: Echinodermata and Lophophorates. *Edited by* A.C. Giese, J.S. Pearse, and V.B. Pearse. Boxwood Press, Pacific Grove, Calif. pp. 513–662.

Pechenik, J.A. 1987. Environmental influences on the larval survival and development. *In* Reproduction of marine invertebrates. Vol. 9. General aspects: seeking unity in diversity. *Edited by* A.C. Giese, J.S. Pearse, and V.B. Pearse. Blackwell Scientific Publications and Boxwood Press, Cambridge. pp. 551–608.

Pennington, J.T. 1985. The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. Biol. Bull. (Woods Hole), **169**: 417–430.

Roller, R.A., and Stickle, W.B. 1985. Effects of salinity on larval tolerance and early developmental rates of four species of echinoderms. Can. J. Zool. **63**: 1531–1538.

Roller, R.A., and Stickle, W.B. 1993. Effects of temperature and salinity acclimation of adults on larval survival, physiology, and early development of *Lytechinus variegatus* (Echinodermata: Echinoidea). Mar. Biol. (Berlin), **116**: 583–591.

Rowley, R.J. 1989. Settlement and recruitment of sea urchins (*Strongylocentrotus* spp.) in a sea-urchin barren ground and a kelp bed: are populations regulated by settlement of post-settlement processes? Mar. Biol. (Berlin), **100**: 485–494.

Rowley, R.J. 1990. Newly settled sea urchins in a kelp bed and urchin barren ground: a comparaison of growth and mortality. Mar. Ecol. Prog. Ser. **62**: 229–240.

Runnström, J. 1918. Heteromorphosen bei Larven von *Parechinus miliaris* und von *Cucumaria frondosa*. Bergens Museums Aarbok No. 15. Bergen, Norway. pp. 1–9.

Runnström, J., and Runnström, S. 1919. Uber die Entwicklung von *Cucumaria frondosa* Gunnerus und *Psolus phantapus* Strussenfeld. Bergens Museums Aarbok No. 5. Bergen, Norway. pp. 1–100.

Rustad, D. 1938. The early development of *Stichopus tremulus* (Gunn.) (Holothuroidea). Bergens Museums. Aarbok No. 8. Bergen, Norway. pp. 1–23.

Rutherford, J.C. 1973. Reproduction, growth and mortality of the holothurian *Cucumaria pseudocurata*. Mar. Biol. (Berlin), **22**: 167–176.

Sloan, N.A. 1979. Microhabitat and resource utilization in cryptic rocky intertidal echinoderms at Aldabra Atoll, Seychelles. Mar. Biol. (Berlin), **54**: 269–279.

Smiley S., McEuen, F.S., Chaffee, C., and Krishnan, S. 1991. Echinodermata: Holothuroidea. *In* Reproduction of marine invertebrates: Echinodermata and Lophophorates. *Edited by* A.C. Giese, J.S. Pearse, and V.B. Pearse. Boxwood Press, Pacific Grove, Calif. pp. 663–750.

Smith, T.B. 1983. Tentacular ultrastructure and feeding behaviour of *Neopentadactyla mixta* (Holothuroidea: Dendrochirota). J. Mar. Biol. Assoc. U.K. **63**: 301–311.

Stickle, W.B., and Diehl, W.J. 1987. Effects of salinity on echinoderms. Echinoderm Stud. **2**: 235–285.

Therriault, J.-C., and Levasseur, M. 1985. Control of phytoplankton production in the lower St. Lawrence Estuary: light and freshwater run-off. Nat. Can. (Que.), **112**: 77–96.

Therriault, J.-C., and Levasseur, M. 1986. Freshwater run-off control of spatio-temporal distribution of phytoplankton in the lower St. Lawrence Estuary (Canada). *In* Proceedings of the NATO Freshwater Sea Workshop, Bodo, Norway. *Edited by* S. Skresled. NATO ASI Ser. Ser. G Ecol. Sci. **7**: 251–260.

Thorson, G. 1946. Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Oresund). Medd. Komm. Dan. Fisk. Havunders. Ser. Plankton, **4**: 1–518.

Watts, S.A., Scheibling, R.E., Marsh, A.G., and McClintock, J.B. 1982. Effect of temperature and salinity on larval development of sibling species of *Echinaster* (Echinodermata: Asteroidea) and their hybrids. Biol. Bull. (Woods Hole), **163**: 348–354.

Yingst, J.Y. 1974. The utilization of organic detritus and associated microorganisms by *Parastichopus parvimemsis*, a benthic deposit-feeding holothurian. Ph.D. dissertation, University of Southern California, Los Angeles, Calif.

Young, C.M., and Chia, F.-S. 1982. Factors controlling spatial distribution of the sea cucumber *Psolus chitonoides*: settling and post-settling behavior. Mar. Biol. (Berlin), **69**: 195–205.

Zann, L., Brodie, J., Berryman, C., and Naqasima, M. 1987. Recruitment, ecology, growth and behavior of juvenile, *Acanthaster planci* (L.) (Echinodermata: Asteroidea). Bull. Mar. Sci. **41**: 561–575.

Distinguishing between populations of fresh- and salt-water harbour seals (*Phoca vitulina*) using stable-isotope ratios and fatty acid profiles

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Abstract: Stable-isotope ratios and fatty acid profiles were used to determine whether the Lacs des Loups Marins harbour seal (*Phoca vitulina mellonae*) on the Ungava peninsula of northern Quebec (160 km east of Hudson Bay) could be distinguished from populations of its marine counterparts, *Phoca vitulina concolor* and *Phoca vitulina richardsi*. *Phoca v. mellonae* had a lower mean $\delta^{13}C$ value than *P. v. concolor* ($p < 0.0001$). Seals from a *P. v. concolor* population from Lake Kasegalik, N.W.T., that have access to both freshwater- and marine-derived carbon had a mean $\delta^{13}C$ value intermediate between that of *P. v. mellonae* and marine *P. v. concolor* and were distinguishable from both. *Phoca v. mellonae*'s blubber contained significantly higher levels of 18:2n-6, 18:3n-3, 20:4n-6, C_{18} polyunsaturated fatty acids, and total polyunsaturated fatty acids than the blubber of harbour seals from two marine populations, and significantly lower levels of 20:1n-9 and 22:1n-11. The n-3/n-6 ratio of *P. v. mellonae* was also significantly lower than that of its marine counterparts. These findings all indicate that the Lacs des Loups Marins harbour seal subsists primarily on a diet derived from freshwater species.

Résumé : On a utilisé des rapports d'isotopes stables et des profils d'acides gras pour déterminer si les phoques communs (*Phoca vitulina mellonae*) du lac des Loups marins, sur la péninsule de l'Ungava, dans le nord du Québec (à 160 km à l'est de la baie d'Hudson), pouvaient être distingués de ceux d'autres populations marines de cet animal, *Phoca vitulina concolor* et *Phoca vitulina richardsi*. Chez les *P. v. mellonae*, on observe une valeur inférieure de $\delta^{13}C$ par rapport à celle des *P. v. concolor* ($p < 0{,}001$). Chez les phoques d'une population des *P. v. concolor* du lac Kasegalik (T.N.-O.), qui ont accès à du carbone provenant de sources dulcicoles et marines, la valeur moyenne de $\delta^{13}C$ était intermédiaire entre celle des *P. v. mellonae* et celle des *P. v. concolor* marins, et les premiers pouvaient être distingués des deux derniers. La graisse de *P. v. mellonae* contenait des concentrations significativement plus élevées de 18:2n-6, de 18:3n-3, de 20:4n-4, d'acides gras polyinsaturés en C_{18} et d'acides gras polyinsaturés totaux que celles de phoques communs des deux populations marines, et des concentrations significativement plus faibles de 20:1n-9 et de 22:1n-11. Le rapport n-3/n-6 des *P. v. mellonae* du lac était également significativement plus faible que celui observé chez les spécimens marins. Ces observations semblent toutes indiquer que les phoques communs du lac des Loups marins subsistent principalement grâce à un régime alimentaire à base d'espèces dulcicoles.
[Traduit par la Rédaction]

Introduction

The harbour seals (*Phoca vitulina mellonae*) of Lacs des Loups Marins or Lower Seal Lakes are found on the Ungava peninsula of northern Quebec, approximately 56–57°N, 73–74°W, 160 km east of Hudson Bay (Smith et al. 1994). The subspecific description of this population is based primarily on an apparently unique morphology and presumed isolation from neighbouring marine harbour seals (Doutt 1942). Though some authors have argued that the supposed morphological anomalies of *P. v. mellonae* are merely artifacts of a small sample, and that the seals are probably able to travel freely between salt and fresh water (Mansfield 1967; Smith and Horonowitsch 1987; see also Honacki et al. 1982; Reeves et al. 1992), other work corroborates the evidence upon which *P. v. mellonae*'s subspecific designation was based (Davies 1958; Smith et al. 1994). Available data indicate that, with the possible exception of harbour seals in Lake Iliamna, Alaska (Everitt and Braham 1980), *P. v. mellonae* is the only known harbour seal population that is resident in freshwater year-round (Consortium Gilles Shooner & Associés et al. 1991*a*).

Phoca v. mellonae's apparently restricted distribution and small population size have prompted the Quebec government to list it as "likely to be designated as threatened or vulnerable" (Quebec Ministère de l'Environnement et de la Faune 1992, p. 86). The International Union for the Conservation of Nature and Natural Resources (IUCN) listed this population as being

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"suspected, but not definitely known, to be endangered, vulnerable, or rare due to a lack of reliable information" (Reijnders et al. 1993, p. 38). Given the uncertainty of *P. v. mellonae*'s status, and the possibility that much of its habitat may be adversely affected by hydroelectric development (Smith et al. 1994), it is important to determine whether it can be distinguished from marine harbour seals using biochemical techniques that have proven effective in other, somewhat similar, investigations. Two such approaches are analyses of stable-carbon and nitrogen isotope ratios in hair (reviewed by Petersen and Fry 1988; see also Lajtha and Michener 1994), and chemometry of fatty acid profiles in the tissue lipids (Grahl-Nielsen et al. 1993; Käkelä et al. 1993).

The stable-isotope approach is based on the fact that stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in animal tissue are ultimately related to diet (DeNiro and Epstein 1978, 1981). We would predict, therefore, that seals from marine food webs would show relatively more ^{13}C in their tissues than those from freshwater food webs. Any enrichment of ^{13}C in *P. v. mellonae* tissue from expected values for freshwater end points would provide evidence of marine input to the diet. Stable-nitrogen isotope ratios in consumer tissues tend to be enriched by about 3‰ compared with dietary values and this stepwise enrichment in ^{15}N in food webs has been used to estimate the trophic levels of animals, including marine mammals (Rau 1982; Minagawa and Wada 1984; Wada et al. 1987; Fry 1988; Hobson and Welch 1992, 1995). When used in conjunction with ^{13}C measurements, stable-nitrogen isotope analysis can provide additional information on the diet of seals as well as their use of marine and freshwater food webs (Hobson and Welch 1992, 1995).

Tissue fatty acid composition is also related to an animal's diet (Hilditch and Williams 1964). Freshwater and marine food webs are characterized by different levels of specific fatty acids, particularly long-chain polyunsaturated fatty acids (PUFA) (Hilditch and Williams 1964). Primary producers in freshwater environments have significantly lower n-3/n-6 ratios than their marine counterparts (Sargent 1976), resulting in freshwater fishes having lower n-3/n-6 ratios, lower levels of C_{20} and C_{22} unsaturated fatty acids, and higher levels of C_{18} PUFA than marine fishes (Henderson and Tocher 1987). In pinnipeds, blubber is the most important site of lipid storage (Pond 1978) and the chemical composition of this tissue is thought to reflect that of the animal's diet (e.g., Iverson et al. 1995). We would expect, therefore, that the fatty acid composition of the blubber in marine seals, which presumably prey exclusively on saltwater fish, would mirror the chemical profile detailed above. Similarly, a freshwater signature in the fatty acid composition of *P. v. mellonae*'s blubber would indicate that it was feeding primarily on freshwater fish species.

We analyzed the tissues of *P. v. mellonae* and two marine populations of harbour seals (*Phoca vitulina richardsi*, eastern Pacific Ocean; *Phoca vitulina concolor*, western Atlantic Ocean and eastern Canadian Arctic) to test the hypothesis that *P. v. mellonae* feeds primarily in a freshwater habitat and is distinguished from its oceanic counterparts by the freshwater signature in its tissue stable-carbon and nitrogen isotope ratios and fatty acid profiles. We also tested whether *P. v. mellonae* was distinguishable from a group of lacustrine *P. v. concolor*, which, because of their proximity to the ocean, probably feed in both freshwater and marine habitats.

Materials and methods

Sample collection

We examined stable-carbon and nitrogen isotope values from three groups of seals: *P. v. concolor* from Kasegalik Lake, Belcher Islands, N.W.T., *P. v. concolor* from the Northwest Atlantic Ocean, and *P. v. mellonae*. Fatty acid values were examined for the latter two groups, in addition to *P. v. richardsi* from the eastern Pacific Ocean.

Most of the hair samples used for stable isotope analyses were garnered from dried pelts in the following collections: the Carnegie Museum, Pittsburgh, Pa. (Table 1; CM accession numbers); the Canadian Museum of Nature, Ottawa, Ont. (CMN accession numbers); and the Greenland Environmental Research Institute, Copenhagen, Denmark (sample PV-G1). The remainder of the samples that were used in both analyses was fresh or frozen tissue collected from animals that were shot (Department of Fisheries and Oceans Canada, Nanaimo Research Station, samples PV-11 through -26; University of Guelph, samples LLM-94-02 and LLM-95-04; Greenland Environmental Research Institute, samples PV-1527, -1612, and -1618), found dead (Grand Manan Whale and Seabird Research Station, North Head, N.B., samples PV-92-01 through -05 and PV-93-01), or drowned incidentally in gill nets (Department of Fisheries and Oceans Canada, Institut Maurice-Lamontagne, samples LLM-89-01 and -02).

Hair and blubber biopsy samples from LLM-94-01, LLM-94-03 through -05, LLM-95-01 through -03, and LLM-95-05 and -06 were collected (by R.J.S.) from live animals that were captured and released in Lacs des Loups Marins in August and September of 1994 and 1995. Biopsies were taken from the animals' side near the pelvis; the area was first frozen with a small injection of lidocaine hydrochloride (Xylocaine, Astra Pharma Inc., Mississauga, Ont.) before the application of a 6 mm diameter biopsy punch (Acu-Punch, Acuderm Inc., Ft. Lauderdale, Fla.).

Potential prey or food-web components collected for stable isotope analysis in Lacs des Loups Marins during 1994 included two lake trout (*Salvelinus namaycush*), four brook trout (*Salvelinus fontinalis*), five lake whitefish (*Coregonus clupeaformis*), and one threespine stickleback (*Gasterosteus aculeatus*).

Stable-isotope analyses

The collected hair samples were washed in detergent using an ultrasonic cleaner and then rinsed repeatedly in distilled water before being dried in an oven at 60°C. Samples were then soaked in ether, rinsed, and air dried before being ground to a powder in a dental amalgam ball mill (Wig-L-Bug). Fish muscle tissue was freeze dried and lipids were extracted using repeated rinses with 2:1 chloroform:methanol. Samples were then air dried and powdered. Samples for $^{13}C/^{12}C$ analysis were loaded into tin cups and combusted in a CN elemental analyser that was part of the Europa Robo-Prep continuous flow mass spectrometer used for ^{13}C analysis of CO_2. Samples for ^{15}N analysis were loaded into quartz tubes with CuO, elemental Cu, Ag wire, and CaO, evacuated, and then sealed using a propane torch. These samples were then combusted at 850°C for 2 h before being allowed to cool slowly. The resultant N_2 gas was introduced directly into an SIRA-12 isotope ratio mass spectrometer. Stable-isotope ratios were expressed using the following notation:

$$\delta X = (R_{sample}/R_{standard} - 1) \times 1000$$

where X is ^{13}C or ^{15}N, R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and the conventional standards were PeeDee Belemnite (PDB) carbonate and atmospheric (AIR) nitrogen for carbon and nitrogen samples, respectively. Analytical error for both $\delta^{13}C$ and $\delta^{15}N$ analyses was estimated at ±0.3‰. ANOVAs ($\alpha = 0.0001$) were used to compare $\delta^{13}C$ and $\delta^{15}N$ values among the groups of seals.

Fatty acid analyses

Immediately after biopsy, blubber samples (approximately 0.5 g each) from LLM-94-01 to -05 were placed in 2 mL of 2:1 chloro-

Table 1. Specimens of *P. v. richardsi*, *P. v. concolor*, and *P. v. mellonae* used in the stable isotope (SI) and fatty acid (FA) analyses.

Specimen	Type of analysis	Location collected	Date collected	Sex
P. v. richardsi				
PV-11	FA	Danger Reefs, B.C.	92-10-02	F
PV-12	FA	Danger Reefs, B.C.	92-10-02	M
PV-13	FA	Snake Island, B.C.	92-07-16	F
PV-14	FA	Nanaimo, B.C.	92-05	F
PV-15	FA	Snake Island, B.C.	92-04-30	F
PV-16	FA	Snake Island, B.C.	92-04-30	F
PV-17	FA	Crofton, B.C.	92-10-06	F
PV-18	FA	Miami Island, B.C.	92-10-06	F
PV-19	FA	North Stuart Channel, B.C.	92-10-06	F
PV-20	FA	North Stuart Channel, B.C.	92-10-06	F
PV-21	FA	Miami Island, B.C.	92-10-06	M
PV-22	FA	North Reef (Crofton), B.C.	92-10-07	M
PV-23	FA	Escape Reef, B.C.	92-10-07	M
PV-24	FA	Thetis Island, B.C.	92-10-07	M
PV-25	FA	Miami Island, B.C.	92-10-07	F
PV-26	FA	Danger Reefs, B.C.	92-10-07	F
P. v. concolor				
PV-92-01	FA	Grand Manan Island, N.B.	92-07-10	M
PV-92-02	SI	Grand Manan Island, N.B.	92-07-28	F
PV-92-03	SI	Grand Manan Island, N.B.	92-08-05	M
PV-92-04	SI	Grand Manan Island, N.B.	92-08-20	F
PV-92-05	SI	Grand Manan Island, N.B.	92-08-20	F
PV-93-01	FA	Grand Manan Island, N.B.	93-09-09	F
PV-1527	FA	Anorluitsoq, Greenland	86-05-01	M
PV-1612	FA	S. Aappilattoq, Greenland	86-05-04	F
PV-1618	FA	Anorluitsoq, Greenland	86-05-11	M
PV-G1	SI	Qaqortoq, Greenland	1993	na
CMN-6138	SI	Nettilling Fiord, Baffin Island	26-03-13	F
CMN-9311	SI	Saguenay Co., Que.	28-06-04	F
CMN-10358	SI	Cape Dorset, Baffin Island	28-10-16	F
CMN-20289	SI	Ha Ha Bay, Que.	50-06-16	M
CMN-20290	SI	Ha Ha Bay, Que.	50-06-15	na
CMN-20291	SI	Ha Ha Bay, Que.	50-06-16	M
CMN-20600	SI	Anticosti Island, Que.	52-06-25	F
CMN-20685	SI	Grand Grève, Que.	51-07-23	F
CMN-20687	SI	Grand Grève, Que.	51-07-23	na
CM-13161	SI	Freshwater Lake, Belcher Islands	37-10-11	na
CM-15217	SI	Kasegalik Lake, Belcher Islands	37-07-15	F (?)
CM-15218	SI	Kasegalik Lake, Belcher Islands	38-06-30	F
CM-15219	SI	Kasegalik Lake, Belcher Islands	38-06-30	F
CM-15220	SI	Kasegalik Lake, Belcher Islands	38-06-30	F
CM-15221	SI	Kasegalik Lake, Belcher Islands	38-07-19	F
CM-17679	SI	La Tabatière, Que.	39-07-01	M
CM-18698	SI	La Tabatière, Que.	39-12-23	F
CM-18745	SI	Lake, Belcher Islands, N.W.T.	Spring 1940	F
CM-18746	SI	Lake, Belcher Islands, N.W.T.	Spring 1940	M
CM-18747	SI	Lake, Belcher Islands, N.W.T.	Spring 1940	M
CM-18748	SI	Lake, Belcher Islands, N.W.T.	Spring 1940	F
CM-19445	SI	Lake, Belcher Islands, N.W.T.	40-08-11	F
P. v. mellonae				
CM-15211	SI	Lacs des Loups Marins, Que.	Summer 1936	F
CM-15212	SI	Lacs des Loups Marins, Que.	Summer 1937	M (?)
CM-15213	SI	Lacs des Loups Marins, Que.	38-03-22	F
CM-15214	SI	Lacs des Loups Marins, Que.	38-03-22	M
CM-15215	SI	Lacs des Loups Marins, Que.	38-03-23	M

Table 1 (*concluded*).

Specimen	Type of analysis	Location collected	Date collected	Sex
CM-17744	SI	Lacs des Loups Marins, Que.	1938–1939	na
LLM-89-01	Both	Lacs des Loups Marins, Que.	89-08	F
LLM-89-02	Both	Lacs des Loups Marins, Que.	89-08	F
LLM-94-01	Both	Lacs des Loups Marins, Que.	94-09-04	F
LLM-94-02	Both	Lacs des Loups Marins, Que.	94-09-05	M
LLM-94-03	Both	Lacs des Loups Marins, Que.	94-09-07	F
LLM-94-04	Both	Lacs des Loups Marins, Que.	94-09-10	F
LLM-94-05	Both	Lacs des Loups Marins, Que.	94-09-11	M
LLM-95-01 (recaptured LLM-94-01)	SI	Lacs des Loups Marins, Que.	95-09-04	F
LLM-95-02	SI	Lacs des Loups Marins, Que.	95-09-07	M
LLM-95-03	SI	Lacs des Loups Marins, Que.	95-09-09	M
LLM-95-04	SI	Lacs des Loups Marins, Que.	95-09-09	F
LLM-95-05	SI	Lacs des Loups Marins, Que.	95-09-11	M
LLM-95-06	SI	Lacs des Loups Marins, Que.	95-09-14	M

form:methanol with 0.005% butylated hydroxytoluene, in Kimax test tubes with teflon caps. Samples were kept at –20°C until further analysis, at which time an additional 7 mL of the chloroform:methanol mixture was added to each tube. Blubber samples from the other seals (Table 1) were taken from fresh carcasses and frozen at –20°C until further analysis. Approximately 0.5 g of each of these samples was placed in 9 mL of the chloroform:methanol solution in Kimax tubes with teflon caps. All samples were mashed manually with a glass homogenizer until thin and transparent, then vortexed for 20 s and allowed to soak for 24 h. Samples (total lipid) were extracted according to a modified Folch procedure (Folch et al. 1957, see Iverson 1988).

Fatty acids of total lipids were converted to methyl esters by placing 50 mg of lipid with 1 mL hexane and 1 mL 10% BF3 in methanol (samples LLM-94-01 to -05), or by placing 50 mg of lipid with 1 mL hexane and 1 mL 10% BF3 in butanol (all other samples) in Kimax tubes, which were then incubated at 100°C for 1 h and allowed to cool to room temperature. Deionized H_2O (3 mL) was added, and the sample was vortexed and centrifuged at 480 × *g* for 5 min. The hexane layer was removed, washed with 5 mL deionized H_2O, and dried with sodium sulphate. Samples were flushed with nitrogen and capped tightly.

Fatty acid composition was determined using temperature-programmed capillary gas–liquid chromatography as described in Iverson et al. (1995) (methyl esters) and in Koopman (1994) (butyl esters). Fatty acids were identified using chromatograms of known standard mixtures (Nu Check Prep, Elysian, Minn.) and from samples earlier identified using silver nitrate chromatography (Iverson 1988). Identified fatty acid components were converted to a mass percentage of the total array of fatty acids plus unknown compounds. The small sample size restricted the number of statistical tests that could be made; thus, only individual fatty acids and fatty acid families that have revealed habitat differences in other species (e.g., see Henderson and Tocher 1987; Käkelä et al. 1993) were compared between groups of seals: 18:2n-6, 18:3n-3, 20:1n-9, 20:4n-6, 22:1n-11, total C_{18} PUFA, total saturates, total monounsaturates, total PUFA, and the n-3/n-6 ratio. The three subpopulations of seals were compared using ANOVA on ranked data (equivalent to a Kruskal–Wallis nonparametric test) with $\alpha = 0.05$.

Results

Stable-isotope analyses

The three sampled populations exhibited different ^{13}C content (ANOVA, $F_{[2,42]} = 149.4$, $p < 0.0001$): *P. v. mellonae* showed the most negative mean $\delta^{13}C$ value, the Kasegalik Lake sample of *P. v. concolor* showed an intermediate value, and the marine population of *P. v. concolor* showed the most positive value (Table 2, Fig. 1). Applying the diet-hair $\delta^{13}C$ fractionation value for seals of +2.8‰ (Hobson et al. 1996), mean dietary $\delta^{13}C$ values for these three populations of seals were calculated as –26.3, –21.9, and –18.9‰ for the *P. v. mellonae*, Kasegalik Lake, and marine *P. v. concolor* samples, respectively.

Stable-nitrogen isotope values of seals from the two freshwater locations were less variable than those from the broader marine sample (Table 2, Fig. 1). Using the seal diet-hair $\delta^{15}N$ fractionation value of +3.0‰ (Hobson et al. 1996), mean dietary $\delta^{15}N$ values of the *P. v. mellonae*, Kasegalik Lake, and marine *P. v. concolor* samples were 9.8, 10.6, and 13.2‰, respectively. Stable-carbon and nitrogen isotope ratios for the sampled Lacs des Loups Marins fish are presented in Table 2.

One freshwater seal that was marked in 1994 (LLM-94-01) was subsequently recaptured in 1995 (LLM-95-01). To avoid analyzing the same animal twice, the means of this animal's 1994 and 1995 $\delta^{13}C$ and $\delta^{15}N$ values (see Fig. 1) were used for the purposes of the ANOVA and the calculation of mean $\delta^{13}C$ and $\delta^{15}N$ values for *P. v. mellonae*.

Fatty acid analyses

The blubber of *P. v. mellonae* contained significantly higher levels of 18:2n-6, 18:3n-3, 20:4n-6, C_{18} PUFA, and total PUFA than did the blubber of either *P. v. concolor* or *P. v. richardsi*. *Phoca v. mellonae* also had significantly lower levels of 20:1n-9 and 22:1n-11 and a lower n-3/n-6 ratio than the two groups of marine seals (Table 3). The most obvious difference between the freshwater and marine seals examined here was the large proportion of n-6 fatty acids in *P. v. mellonae*'s blubber. Levels of total n-3 fatty acids in these animals were also higher than those of both marine seal groups, but were closer to the range of values observed for marine seals than were the levels of n-6 fatty acids (Fig. 2).

There were some significant differences between the fatty acid composition of the blubber of the two groups of marine seals: *P. v. concolor* (northwest Atlantic) had higher levels of

Table 2. Stable-carbon and nitrogen isotope values (mean ± SD) of hair from seals and muscle tissue from Lacs des Loups Marins fish.

Sample	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Seals			
P. v. mellonae	18	–23.5±1.4	12.8±0.9
P. v. concolor (Kasegalik Lake)	11	–19.1±1.0	13.6±1.2
P. v. concolor (marine)	16	–16.1±1.2	16.2±3.4
Lacs des Loups Marins fish			
Salvelinus sp.	6	–23.5±1.4	11.1±0.8
Coregonus clupeaformis	5	–21.4±0.7	8.7±0.7
Gasterosteus aculeatus	1	–26.3	10.7

Table 3. Levels of selected fatty acids in the blubber of three subspecies of harbour seal (*Phoca vitulina*).

	P. v. concolor (marine)	*P. v. richardsi* (marine)	*P. v. mellonae* (freshwater)
Fatty acids			
18:2n-6	1.29±0.08*a*	1.06±0.15*b*	4.78±0.84*c*
18:3n-3	0.52±0.12*a*	0.44±0.11*a*	2.88±0.78*b*
20:1n-9	8.77±1.90*a*	2.34±0.92*b*	0.09±0.16*c*
20:4n-6	0.40±0.17*a*	0.54±0.15*b*	2.69±0.20*c*
22:1n-11	3.42±1.78*a*	0.63±0.44*b*	0.02±0.01*c*
Total saturates	15.73±1.36*a*	14.54±3.67*a*	12.07±1.43*a*
Total monounsaturates	58.80±3.76*ab*	63.15±5.97*a*	49.60±6.38*b*
Total PUFA	23.36±2.59*a*	19.94±5.30*a*	34.26±6.39*b*
Total C_{18} PUFA	3.94±0.30*a*	2.81±0.43*b*	10.39±2.02*c*
n-3/n-6 ratio	7.65±0.95*a*	6.31±1.36*b*	2.05±0.29*c*

Note: Levels of fatty acids are given as percent wet weight (± standard deviation) of total fatty acids and unknowns present in the sample. Levels of each fatty acid in a subspecies followed by different letters were significantly different ($p < 0.05$). Sample sizes were 5, 16, and 7 for *P. v. concolor*, *P. v. richardsi*, and *P. v. mellonae*, respectively.

18:2n-6, 20:1n-9, 22:1n-11, and C_{18} PUFA and a larger n-3/n-6 ratio in their blubber than did *P. v. richardsi* (eastern Pacific).

Discussion

Results from the stable-isotope and fatty acid analyses both indicated that the diet of Lacs des Loups Marins seals is principally of freshwater origin.

Stable-carbon isotope ratios of primary producers in freshwater aquatic food webs are typically less enriched in ^{13}C than those of marine food webs and this difference is passed on to higher order consumers (Fry and Sherr 1988). On the basis of this difference in source ^{13}C, stable-carbon isotope analysis has been used successfully to delineate the relative dependence of consumers on carbon from each of these biomes (Chisholm et al. 1982; Hobson 1990; Hobson and Sealy 1991; Ramsay and Hobson 1991). Stable-carbon isotope analysis of seal hair allowed us to successfully segregate the three populations of seals from freshwater and marine habitats (Fig. 1). As predicted, *P. v. mellonae* showed the most depleted $\delta^{13}C$ values, which is consistent with a diet derived from freshwater prey during the period when the hair was grown. The fact that

Fig. 1. Distribution of stable-carbon and nitrogen isotope ratios in the hair of harbour seals with diets derived from marine (*P. v. concolor*; Northwest Atlantic), freshwater and marine (*P. v. concolor*; Kasegalik Lake), and freshwater (*P. v. mellonae*) sources. Subscripts 1 and 2 denote the 1994 and 1995 values, respectively, for the one freshwater seal that was marked and recaptured.

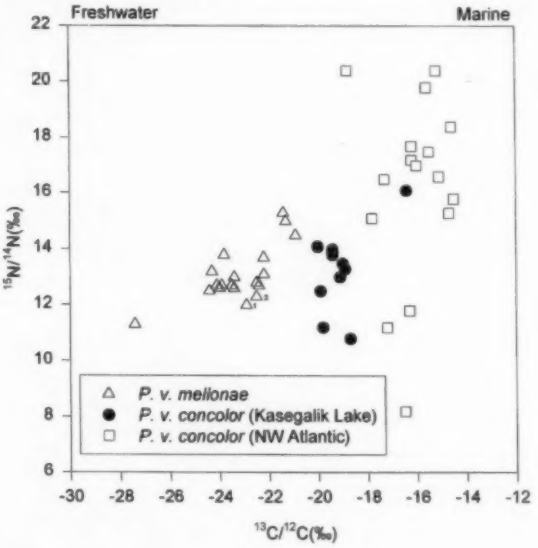
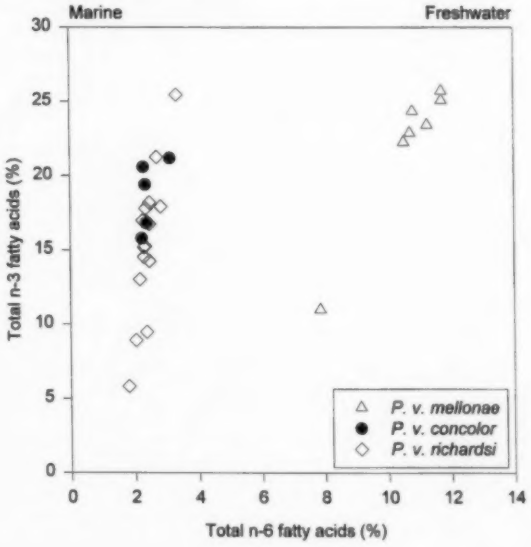


Fig. 2. Plot of total n-3 versus total n-6 fatty acids (as percentage of total fatty acids) in the blubber of marine (*P. v. richardsi* and *P. v. concolor*) and freshwater (*P. v. mellonae*) harbour seals.



there was no discernible difference between the isotopic signatures of the *P. v. mellonae* samples from 1994 and 1995, and that the marked and recaptured animal (LLM-94-01, LLM-95-

01) had a $\delta^{13}C$ value that was virtually identical in both years (–22.9 in 1994, –22.5 in 1995), is further evidence of freshwater feeding.

While few isotopic studies have been conducted on fish in Arctic drainages, Hesslein et al. (1989, 1991) demonstrated that variation in isotope values among species can indicate different sources of feeding both within and outside of local lakes. Our $\delta^{13}C$ values for trout in Lacs des Loups Marins overlap those found for char (*Salvelinus alpinus*) at Char Lake, N.W.T. (Hobson and Welch 1995). Whitefish and stickleback values are similar to those found by Hesslein et al. (1991) in Travaillant Lake, N.W.T.

The lake whitefish and trout populations in Lacs des Loups Marins are resident populations (Consortium Gilles Shooner & Associés et al. 1991*b*), and limited evidence from stomach contents and scat analyses indicates that *P. v. mellonae*'s diet consists, in large part, of these fish species (Consortium Gilles Shooner & Associés et al. 1991*a*; R.J. Smith, unpublished data). Given that the $\delta^{13}C$ values of the trout and whitefish sampled from Lacs des Loups Marins were more depleted than those calculated for the mean seal diet, it is clear that a more extensive carbon isotopic survey of potential prey of *P. v. mellonae* is required before any further inferences can be made about the composition of seal diets within Lacs des Loups Marins.

Phoca v. concolor from northwest Atlantic marine populations showed $\delta^{13}C$ values that are typical of marine mammals measured elsewhere in north-temperate regions (Ramsay and Hobson 1991; Hobson and Welch 1992; Muir et al. 1996). The Kasegalik Lake sample was intermediate in $\delta^{13}C$ signature between the marine and Lacs des Loups Marins values. Kasegalik Lake is, at some points, less than 2 km from the ocean, and the Arctic char that frequent the lake are anadromous (Sanikiluaq Hamlet Council 1978). Thus, seals collected from Kasegalik Lake had access to both freshwater and marine-derived carbon, either because of their use, or because of their prey's use, of both kinds of aquatic habitat.

Our stable-nitrogen isotope values for the marine *P. v. concolor* sample indicated that it occupied at least two trophic levels. This is consistent with published stomach content and scat analyses that have indicated a broad diet consisting of invertebrates as well as planktivorous and omnivorous fish (Boulva and McLaren 1979; Payne and Selzer 1989). The *P. v. mellonae* and Kasegalik Lake *P. v. concolor* samples were similar in $\delta^{15}N$ values, suggesting that they occupy similar trophic positions. The estimated mean $\delta^{15}N$ values of 10.3–10.6‰ for the prey of freshwater seals are in close agreement with those values found for fish in Lacs des Loups Marins (Table 2). Freshwater seals also showed less variation in trophic position, probably because of fewer dietary alternatives in high-latitude lakes compared with Arctic marine systems (e.g., Kling et al. 1992; Hobson and Welch 1992, 1995).

The fatty acid composition of the blubber of *P. v. mellonae* also indicated that the lipid in these seals is derived from a freshwater source. The plot of n-3 versus n-6 fatty acids (Fig. 2) shows that levels of n-6 alone can distinguish the freshwater harbour seals from the two marine populations. The pattern of high levels of n-6 and C_{18} PUFA and low levels of monounsaturated C_{20} and C_{22} in the Lacs des Loups Marins seals is typical of the fatty acid composition of organisms in freshwater environments (Henderson and Tocher 1987). Henderson and Tocher (1987) noted that the n-3/n-6 ratio in freshwater fish ranges from 0.5 to 3.8, while in marine fish the value is typically between 4.7 and 14.4. Assuming similar ratios for higher level consumers like seals, this further indicates that *P. v. mellonae* (ratio 2.05) is feeding in fresh water, whereas *P. v. concolor* (ratio 7.65) and *P. v. richardsi* (ratio 6.31) are consuming marine prey.

There are few comparative studies of the fatty acid composition of other mammals in freshwater environments; however, those that do exist have arrived at conclusions similar to those of the present investigation. Käkelä et al. (1993) examined the fatty acid composition of the blubber of Saimaa ringed seals (*Phoca hispida saimensis*) from a freshwater lake and found that it contained higher levels of 20:4n-6 (3.02%) than did the blubber of ringed seals (*Phoca hispida botnica*) from the Baltic (0.63%). However, these authors found the levels of 18:2n-6 and the n-3/n-6 ratio in the blubber of Saimaa seals (4.50% and 4.58, respectively) to be the same as those of the Baltic seals (4.43% and 4.53, respectively) and suggested the presence of a gradient of fatty acids from freshwater to brackish to marine food webs. Käkelä et al. (1995) found the levels of C_{22} n-6 fatty acids in the blubber of freshwater ringed seals from lakes Saimaa and Ladoga to be twice as high as those in marine ringed seals from the Arctic Ocean. In our study, the separation between marine and freshwater seals on the basis of these fatty acids was even clearer; the blubber of the marine seals contained less than one-tenth the amount of C_{22} n-6 fatty acids observed in the freshwater seals (*P. v. richardsi*, 0.18%; *P. v. concolor*, 0.13%; *P. v. mellonae*, 1.95%). The Amazon river dolphin (*Inia geoffrensis*) is completely riverine and its blubber is characterized by an extremely low n-3/n-6 ratio (0.27), which is primarily the result of high levels of 18:2n-6 (6.61%) (Ackman et al. 1971). *Inia geoffrensis* blubber also contains low levels of 20:1 (0.52%) (Ackman et al. 1971), similar to those observed here in *P. v. mellonae* (0.61%).

The two marine harbour seal subspecies we examined exhibited clear marine signatures in the fatty acid patterns of their blubber. Surveys of the composition of the blubber of five phocid seal species revealed n-3/n-6 ratios ranging from 9 to 24, and levels of 20:1 and 22:1 ranging from 5.0 to 16.6 and from 0.7 to 6.5, respectively (Engelhardt and Walker 1974; West et al. 1979). While the fatty acid compositions of the blubber of *P. v. concolor* and *P. v. richardsi* are similar to those of other marine seals, they can also be distinguished from one another on the basis of levels of monounsaturated C_{20} and C_{22}. These fatty acids originate in marine copepods (Ackman 1980), and the significantly higher levels of these compounds in the *P. v. concolor* sample (Table 3) are probably a reflection of variation in the diet of the primary prey species in each area. Levels of different isomers of monounsaturated C_{22} fatty acids can also be used to provide further evidence that the Lacs des Loups Marins seals are feeding in freshwater; 22:1n-11 originates in marine copepods, while 22:1n-9 is found in freshwater invertebrates (Ratnayake and Ackman 1979; Bell et al. 1994). The ratio of 22:1n-11 to 22:1n-9 in the *P. v. mellonae* seals (0.56) was much lower than in the *P. v. richardsi* and *P. v. concolor* seals (7.52 and 8.21, respectively), and 22:1n-11 was virtually absent in the freshwater seals, suggesting that the Lacs des Loups Marins animals are not feeding in a marine food web.

Our study has demonstrated the utility of two relatively

new analytical techniques for the study of marine mammals with access to marine or freshwater biomes. While our analyses were performed on samples that did not allow year-round inferences of seal diet, they do provide additional evidence that *P. v. mellonae* is resident in freshwater and point to interesting future research possibilities in this unique aquatic ecosystem. Further isotopic analysis of bone collagen would, for example, provide dietary inferences based on the lifetime of individuals (Tieszen and Boutton 1988). In addition, isotopic and fatty acid analysis based on samples collected throughout the year would reveal seasonal dietary patterns. We are currently engaged in a larger isotopic study to characterize the trophic structure of Lacs des Loups Marins to better understand the role of a pinniped predator in such a system. The results of this work are also relevant to the ongoing debates concerning hydroelectric development on the Ungava peninsula and their potential impact on the resident pinniped population of the Lacs des Loups Marins area.

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References

Ackman, R.G. 1980. Fish lipids. Part 1. *In* Advances in fish science and technology. *Edited by* J.J. Connell. Fishing News Books, Surrey, England. pp. 86–103.

Ackman, R.G., Eaton, C.A., and Litchfield, C. 1971. Composition of wax esters, triglycerides and diacyl glyceryl ethers in the jaw and blubber fats of the Amazon river dolphin (*Inia geoffrensis*). Lipids, **6**: 69–77.

Bell, J.G., Ghioni, C., and Sargent, J.R. 1994. Fatty acid compositions of 10 freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar*): a comparison with commercial diets. Aquaculture, **128**: 301–313.

Boulva, J., and McLaren, I.A. 1979. Biology of the harbor seal, *Phoca vitulina*, in eastern Canada. Bull. Fish. Res. Board Can. No. 200.

Chisholm, B.S., Nelson, D.E., and Schwarcz, H.P. 1982. Stable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science (Washington, D.C.), **216**: 1131–1132.

Consortium Gilles Shooner & Associés, Somer, and Environnement Illimité Inc. 1991*a*. Complexe Grande-Baleine. Avant-projet phase 2. Bilan des connaissances sur le phoque d'eau douce. Vice-Présidence Environnement, Hydro-Québec, Montréal, Que.

Consortium Gilles Shooner & Associés, Somer, and Environnement Illimité Inc. 1991*b*. Complexe Grande-Baleine. Avant-projet phase 2. Caractérisation des périodes et des habitats de reproduction de la faune ichtyenne. Vice-Présidence Environnement, Hydro-Québec, Montréal, Que.

Davies, J.L. 1958. Pleistocene geography and the distribution of northern pinnipeds. Ecology, **39**: 97–113.

Deniro, M.J., and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta, **42**: 495–506.

Deniro, M.J., and Epstein, S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta, **45**: 341–351.

Doutt, J.K. 1942. A review of the genus *Phoca*. Ann. Carnegie Mus. **29**: 61–125.

Engelhardt, F.R., and Walker, B.L. 1974. Fatty acid composition of the harp seal, *Pagophilus groenlandicus* (*Phoca groenlandica*). Comp. Biochem. Physiol. B, **47**: 169–179.

Everitt, R.D., and Braham, H.W. 1980. Aerial survey of Pacific harbor seals in the southeastern Bering Sea. Northwest Sci. **54**:281–288.

Folch, J., Lees, M., and Sloan-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. **226**: 497–509.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N and S isotopic compositions. Limnol. Oceanogr. **33**: 1182–1190.

Fry, B., and Sherr, E.B. 1988. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *In* Stable isotopes in ecological research. *Edited by* P.W. Rundel, J.R. Ehleringer, and K.A. Nagy. Springer Verlag, New York. pp. 196–229.

Grahl-Nielsen, O., Mjaavatten, O., and Tvedt, D.E. 1993. Distinguishing between different populations of harp seal (*Phoca groenlandica*) by chemometry of the fatty acid profiles in the jaw bone. Can. J. Fish. Aquat. Sci. **50**: 1400–1404.

Henderson, R.J., and Tocher, D.R. 1987. The lipid composition and biochemistry of freshwater fish. Prog. Lipid Res. **26**: 281–347.

Hesslein, R.H., Fox, D.E., and Capel, M.J. 1989. Sulfur, carbon, and nitrogen isotopic composition of fish from the Mackenzie River delta region and other Arctic drainages. Can. Data Rep. Fish. Aquat. Sci. No. 728.

Hesslein, R.H., Capel, M.J., Fox, D.E., and Hallard, K.A. 1991. Stable isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River basin, Canada. Can. J. Fish. Aquat. Sci. **48**: 2258–2265.

Hilditch, T.P., and Williams, P.N. 1964. The chemical constitution of natural fats. 4th ed. Chapman and Hall, London.

Hobson, K.A. 1990. Stable isotope analysis of marbled murrelets: evidence for freshwater feeding and determination of trophic level. Condor, **92**: 897–903.

Hobson, K.A., and Sealy, S.G. 1991. Marine protein contributions to the diet of Northern Saw-whet Owls on the Queen Charlotte Islands: a stable isotope approach. Auk, **108**: 437–440.

Hobson, K.A., and Welch, H.E. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar. Ecol. Progr. Ser. **84**: 9–18.

Hobson, K.A., and Welch, H.E. 1995. Cannibalism and trophic structure in a high Arctic lake: insights from stable-isotope analysis. Can. J. Fish. Aquat. Sci. **52**: 1195–1201.

Hobson, K.A., Schell, D.M., Renouf, D., and Noseworthy, E. 1996. Stable-carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary

reconstructions involving marine mammals. Can. J. Fish. Aquat. Sci. In press.

Honacki, J.H., Kinman, K.E, and Koeppl, J.W. 1982. Mammal species of the world. Allen Press and the Association of Systematics Collections, Lawrence, Kans.

Iverson, S.J. 1988. Composition, intake, and gastric digestion of milk lipids in pinnipeds. Ph.D. thesis, University of Maryland, College Park, Md.

Iverson, S.J., Oftedal, O.T., Bowen, W.D., Boness, D.J., and Sampugna, J. 1995. Prenatal and postnatal transfer of fatty acids from mother to pup in the hooded seal. J. Comp. Physiol. B, **165**: 1–12.

Käkelä, R., Hyvärinen, H., and Vainiotalo, P. 1993. Fatty acid composition in liver and blubber of the Saimaa ringed seal (*Phoca hispida saimensis*) compared with that of the ringed seal (*Phoca hispida botnica*) and grey seal (*Halichoerus grypus*) from the Baltic. Comp. Biochem. Physiol. B, **105**: 553–565.

Käkelä, R., Ackman, R.G., and Hyvärinen, H. 1995. Very long chain polyunsaturated fatty acids in the blubber of ringed seals (*Phoca hispida* sp.) from Lake Saimaa, Lake Ladoga, the Baltic Sea, and Spitsbergen. Lipids, **30**: 725–731.

Kling, G.W., Fry, B., and O'Brien, W.J. 1992. Stable isotopes and planktonic trophic structure in arctic lakes. Ecology, **73**: 561–566.

Koopman, H.N. 1994. Topographical distribution and fatty acid composition of blubber in the harbour porpoise, *Phocoena phocoena*. M.Sc. thesis, Department of Zoology, University of Guelph, Guelph, Ont.

Lajtha, K., and Michener, R.H. 1994. Stable isotopes in ecology and environmental science. Blackwell Scientific Publications, Oxford.

Mansfield, A.W. 1967. Distribution of the harbor seal, *Phoca vitulina* Linnaeus, in Canadian arctic waters. J. Mammal. **48**: 249–257.

Minagawa, M., and Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim. Cosmochim. Acta, **48**: 1135–1140.

Muir, D.C.G., Segstro, M.D., Hobson, K.A., Ford, C.A., Stewart, R.E.A., and Olpinski, S. 1996. Can elevated levels of PCBs and organochlorine pesticides in walrus blubber from eastern Hudson Bay (Canada) be explained by consumption of seals? Environ. Pollut. In press.

Payne, P.M., and Selzer, L.A. 1989. The distribution, abundance and selected prey of the harbor seal, *Phoca vitulina concolor*, in southern New England. Mar. Mamm. Sci. **5**: 173–192.

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18**: 293–320.

Pond, C.M. 1978. Morphological aspects and the ecological and mechanical consequences of fat deposition in wild vertebrates. Annu. Rev. Ecol. Syst. **9**: 519–570.

Quebec, Ministère de l'Environnement et de la Faune. 1992. Liste des espèces de la faune vertébrées susceptibles d'être désignées menacées ou vulnérables. Ministère de l'Environnement et de la Faune, Quebec.

Ramsay, M.A., and Hobson, K.A. 1991. Polar bears make little use of terrestrial food webs: evidence from stable-carbon isotope analysis. Oecologia, **86**: 598–600.

Ratnayake, W.N., and Ackman, R.G. 1979. Fatty alcohols in capelin, herring and mackerel oils and muscle lipids. I. Fatty alcohol details linking dietary copepod fat with certain fish depot fats. Lipids, **14**: 795–803.

Rau, G.H. 1982. The relationship between trophic level and stable isotopes of carbon and nitrogen. *In* Coastal water research project biennial report for the years 1981–1982. *Edited by* W. Bascom. Southern California Water Research Project, Long Beach, Calif. pp. 143–148.

Reeves, R.R., Stewart, B.S., and Leatherwood, S. 1992. The Sierra Club handbook of seals and sirenians. Sierra Club Books, San Francisco, Calif.

Reijnders, P.J.H., Brasseur, S., Van Der Toorn, J., Van Der Wolf, P., Boyd, I., Harwood, J., Lavigne, D., Lowry, L., and Stuart, S. (*Editors*). 1993. Seals, fur seals, sea lions and walruses: status of pinnipeds and conservation action plan. International Union for the Conservation of Nature and Natural Resources (IUCN), Gland, Switzerland.

Sanikiluaq Hamlet Council. 1978. The Belcher Island char fishery: a statement of concern. Sanikiluaq Hamlet Council. Available from Department of Fisheries and Oceans, Box 358, Iqaluit, NT X0A 0H0, Canada.

Sargent, J.R. 1976. The structure, metabolism and function of lipids in marine organisms. *In* Biochemical biophysical perspectives in marine biology. Vol. 3. *Edited by* D.C. Malins and J.R. Sargent. Academic Press, New York. pp. 149–212.

Smith, R.J., Lavigne, D.M., and Leonard, W.R. 1994. Subspecific status of the freshwater harbor seal (*Phoca vitulina mellonae*): a re-assessment. Mar. Mamm. Sci. **10**: 105–110.

Smith, T.G., and Horonowitsch, G. 1987. Harbour seals in the Lacs des Loups Marins and eastern Hudson Bay drainage. Can. Tech. Rep. Fish. Aquat. Sci. No. 1539.

Tieszen, L.L., and Boutton, T.W. 1988. Stable carbon isotopes in terrestrial ecosystem research. *In* Stable isotopes in ecological research. *Edited by* P.W. Rundel, J.R. Ehleringer, and K.A. Nagy. Springer Verlag, New York. pp. 167–195.

Wada, E., Terazaki, M., Kabaya, Y., and Nemoto, T. 1987. ^{15}N and ^{13}C abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. Deep-Sea Res. **34**: 829–841.

West, G.C., Burns, J.J., and Modafferi, M. 1979. Fatty acid composition of blubber from the four species of Bering Sea phocid seals. Can. J. Zool. **57**: 189–195.

Anatomical and physiological factors affecting the swim-bladder cross-section of the sardine *Sardina pilchardus*

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Abstract: Variations in the geometric cross-section of the swim-bladder of the sardine *Sardina pilchardus*, at different reproductive stages, were examined in relation to fish length, the relative volume of gonads and stomach, and lipid content. Fifty specimens were frozen in liquid nitrogen and sectioned with a cryomicrotome, and the volume and surface measurements of their organs were obtained by computer reconstruction. Equations that calculate the swim-bladder cross-section from the aforementioned variables are given. The contribution of each variable to the variations in the cross-section size and shape was estimated by means of multiple regression analysis. We concluded that during the nonreproductive period, the main variable affecting cross-section is length, whereas during the reproductive period, the effect of gonads is more important than length in the calculation of the swim-bladder cross-section. Consequently, variables other than length should be taken into account when estimating target strength, as the latter is affected by the size of the swim-bladder cross-section.

Résumé : On a étudié les variations géométriques de la section transversale de la vessie natatoire de la sardine *Sardina pilchardus* en rapport avec la longueur du poisson, les volumes relatifs des gonades et de l'estomac et la teneur en lipides, à différentes étapes du processus reproductif. Cinquante spécimens ont été congelés dans l'azote liquide et découpés au microtome, et on a obtenu des mesures du volume et de la surface de leurs organes par reconstruction informatisée. On présente les équations permettant de calculer la section transversale de la vessie natatoire à partir de ces variables. La contribution de chacune des variables aux variations de l'aire et de la forme de la section transversale a été estimée à l'aide d'une analyse de régression multiple. Nous avons conclu que, au cours de la période non reproductive, la principale variable influant sur la section transversale est la longueur, alors que, pendant la période de la reproduction, l'effet des gonades est plus important que celui de la longueur pour le calcul de la section transversale de la vessie natatoire. Par conséquent, il faut prendre en considération d'autres variables que la longueur pour l'estimation de l'effectif de l'espèce étudiée, étant donné que l'aire de la section transversale de la vessie natatoire influe sur cette dernière.
[Traduit par la Rédaction]

Introduction

The fish backscattering cross-section, or target strength (TS), is a pivotal quantity in the acoustic assessment of fish abundance. It is generally agreed that the swim-bladder is the major component of the backscattering echo of a fish, contributing more than 90–95% to the total echo (Foote 1980, 1985). Consequently, quantitative estimates of fish abundance using an echo sounder could be substantially affected by changes in the size and shape of the swim-bladder (Foote 1980, 1985; Blaxter and Batty 1990). Furthermore, TS changes with respect to fish orientation (aspect) in the acoustic beam. This is because backscattering energy is proportional to the geometric cross-sectional area of the object that scatters the echo (Clay and Medwin 1977).

Target strength has been the focus of numerous and diverse studies (Foote 1985; MacLennan and Simmonds 1992). These have included in situ measurements, controlled measurements on tethered or encased fish, and modeling. To date, all TS measurements have been related only to fish length. However, it has been hypothesized that other factors may also contribute to the variability in TS measurements (MacLennan and Simmonds 1992). There are no data that relate fish TS or swim-bladder geometric cross-sectional area to factors other than fish length, nor has the possible influence of such factors ever been quantified. Ona (1990) provided a qualitative demonstration of gadoid swim-bladder changes in relation to fish lipid content, gonad size, and stomach fullness, surmising as to the corresponding changes in TS. Buerkle (1987) suggested that the unexplained variation (25.9%) of his cod TS measurements might be attributed to such factors.

The present work examines the variations in the maximum geometric cross-sectional area of the swim-bladder of the sardine *Sardina pilchardus* (Walbaum 1792) relative to fish length, gonad volume, stomach volume, and lipid content. We provide qualitative evidence that the aforementioned organs and lipid tissue influence the cross-sectional area of the swim-bladder, and estimate their relative importance for sardines at

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different stages of maturity. We further demonstrate that in certain instances gonads can be more important than length in predicting swim-bladder geometric cross-sectional area, and infer similar effects on the TS.

Materials and methods

Fish collection

The sardine was chosen because of its small size (which made manipulation easier) and because it is characterized by distinct seasonal variations in physiology (Culley 1970). Five samples of live sardines were collected from July 1989 to July 1990, using a small purse seine. This fishing gear minimized mortality caused by injuries to the extremely sensitive skin of the sardine. The five samples were collected in July 1989 (summer 1989), mid–October 1989 (autumn), late December 1989 (winter), late April 1990 (spring), and July 1990 (summer 1990). The fish were transported in plastic bags filled with water (Verheijen 1955) and placed in 417-L cylindrical tanks, with smooth black walls and a constant flow of seawater. Fish densities in the tanks were kept at 0.04–0.63 fish/L. To prevent infection, the fish were immersed in a bath of antibiotic (Illeganine 100 – Furaltadine – HCl 100%, 35 ppm), for 1 h/d, by stopping the water flow and adding the solution. Mortality was reduced to zero by the 3rd d of captivity. Following a period of acclimatization (4–5 d), a subsample of 10 fish was taken from each sample for laboratory analysis.

Anesthesia and freezing

Each fish was anaesthetized with MS-222 (1 : 20 000) (Muzinic 1970), and the weight and standard length of each fish were measured. Immediately after immobilization, the fish were transferred to liquid nitrogen. During the freezing procedure, each fish was held in the normal, horizontal position, stretched between a pair of tongs grasping the snout and tail. With this method the fish were deep frozen within 30–50 s. The specimens were kept at –50°C until processed (Farrant et al. 1977; Ona 1990). As Ona (1990) pointed out, this method of freezing and slicing permits the actual swim-bladder volume to be estimated with a positive bias of no more than 0.1%, while more than 95% of the observed deformations are real.

Modeling and slicing

The fish were first embedded in blocks of carboxymethylcellulose (CMC), accomplished by immersing the frozen fish in an aqueous water solution of 3% CMC held in the microtome's freezing frame. This was immediately followed by rapid freezing of the entire system by immersion in liquid nitrogen. Each specimen was sliced with a cryomicrotome (Reichert-Jung HN40, nominal accuracy 1 µm). Slicing was done in the sagittal plane to minimize the number of sections (Foote and Ona 1985; Ona 1990). Each block was sliced at 50-µm intervals and photographed at 150-µm intervals. A stable immobile parallelogram, of known dimensions, was used as an absolute reference (reference parallelogram) for the three-dimensional reconstruction of the swim-bladder cavity, gonads, and stomach (Ona 1990).

The slices of the fish were collected and dehydrated by freeze-drying, and the dry weight of each fish was measured (Henderson and Almatar 1989). The total fat content was estimated using the Soxhlet method for lipid extraction with petroleum ether at 50–70° (Hickling 1945).

Reconstruction

Three-dimensional reconstruction was performed by digitizing (from photographed sagittal sections) the inner contour of the wall of the swim-bladder and the outline of the gonads and stomach, together with its reference system. For these procedures, the software image analysis OPRS (Optical Recognition System) was used.

Digitized cross-sectional contours for each fish, scaled to true size and the actual depth position of each slice, provided the data needed for computer-generated reconstruction of the swim-bladder, gonads, and stomach. This point-to-point reconstruction was further used to calculate the volume of the swim-bladder, gonads, and stomach, and the maximum swim-bladder backscatter surface (Foote and Ona 1985). The process of reconstruction and the relevant calculations were performed using the software Slicer developed by the Institute of Marine Biology of Crete. The software measured organ volume by volume integration. The area of each slice and the volume (V) of each organ were calculated according to the following equation:

$$[1] \qquad V = \frac{1}{3} \cdot h \cdot (E_1 + E_k + \sum_{i=1}^{i=k-1} (E_i + \sqrt{E_i \cdot E_{i+1}} + E_{i+1}))$$

where k is the number of slices, E_i is the surface area of the i^{th} slice, and h is 150 µm, the distance between two successive photographed slices (Culling et al. 1985).

All of the points of the swim-bladder were projected on a horizontal plane. The orientation of this plane was determined by three landmarks: the end of the snout and the two retinas (all of which had also been digitized). The position of these landmarks, especially the two retinas, can be defined with minimum variance and using them provides a stable orientation of the plane with respect to the fish. The two retina landmarks define an axis vertical to the longitudinal axis of the fish. Other points, such as the tip of the tail, are much more difficult to define repeatedly with the same precision.

From the projected points the cross-sectional area and perimeter of the swim-bladder were calculated. The initial plane was rotated around the axis fixed by the two retinas, from 0 to 180°, in 1° steps. In each step, the projected surface area of the new plane was calculated. Through this procedure the maximum geometric cross-sectional area of the swim-bladder and its tilt angle with respect to the initial plane were determined. The cross-sectional area is well defined and can be used to compare fish of different lengths and physiological condition. Such is also the case for TS measurements: the TS changes considerably over a small range of tilt angles for fish that are small in relation to the wavelengths used in acoustic surveys. Moreover, there is a well-defined maximum in dorsal aspect TS with respect to tilt angle. Different species and sizes of fish can also be compared in terms of maximum TS (Love 1969, 1977). This TS closely corresponds to the swim-bladder angle in relation to the fish's longitudinal axis (Blaxter and Batty 1990).

The perimeter was used to calculate the circularity of the shape: $C_s = P^2/A$, where P is the perimeter of the swim-bladder cross-section and A is the maximum surface area. C_s tends to a minimum of 4π as the shape approximates a circle. C_s was used to study the changes in the shape of the maximum swim-bladder cross-section (Squire 1978).

Statistical analysis

To minimize heteroscedasticity caused by mortality, specimens to be analyzed were chosen so that all length classes of the original catch were included. Preliminary correlation analysis showed that the most powerful (noncolinear) explanatory variables were (*i*) the percentage of gonad volume in relation to the wet weight of the fish (relative gonad volume), (*ii*) the percentage of stomach volume in relation to the wet weight of the fish (relative stomach volume), (*iii*) the percentage of total lipid weight in relation to the dry weight of the fish (relative lipid content), and (*iv*) the standard length of the fish. These variables introduce the uncorrelated influence of gonad volume, stomach volume, and lipid content on swim-bladder cross-section, after accounting for the influence of fish size. To examine the influence of sex, a dummy variable (D_s) was introduced with a value of 0 for males and 1 for females. Another dummy variable (D_g) was used in the annual regression with a value of 1 for the reproductive and 0 for the nonreproductive period. For each sample a specific seasonal equation was estimated.

The above-mentioned variables, together with the swim-bladder volume, were used to calculate the Mahalanobis distances. Further,

Table 1. The ranges of the variables for each sample.

	Length (cm)		Gonad (%)		Stomach (%)		Lipid (%)		Area (cm^2)	
Sample	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Summer 1989	10.2	15.1	0.01	0.61	0.4	1.2	8.3	37.2	1.7	4.2
Summer 1990	13.0	16.0	0.12	0.79	0.5	0.9	25.8	46.2	1.5	3.8
Spring	14.0	15.8	0.48	1.66	0.5	0.8	12.9	26.2	2.1	4.3
Autumn	13.5	16.3	1.40	5.04	0.3	0.6	25.1	47.8	1.2	3.0
Winter	14.6	16.4	5.03	9.37	0.6	1.2	4.3	36.6	2.0	4.5

Note: Length, standard length; gonad, percentage of gonad volume in terms of the wet weight of the fish; stomach, percentage of stomach volume in terms of the wet weight of the fish; lipids, percentage of lipid weight in terms of the dry weight of the fish; area, the maximum geometric cross-sectional area of the swim-bladder.

the samples were matched by cluster analysis to estimate interseasonal relationships.

Forward stepwise multiple regression analysis was used to estimate the combined influence of the biological factors on the cross-sectional area. This method is a useful device to sort out the relative importance of the independent variables in explaining the variance of the dependent variable. This was achieved by choosing the sequence of regressors (e.g., the mathematical expression of variables) that will maximize $\overline{R}^2$ (adjusted R^2). The coefficient of multiple determination (R^2) provides an overall index of how well Y (the dependent variable) can be explained by the regressors (the independent variables). In addition, the difference between successive R^2 values shows the independent contribution made by each additional regressor to the overall explanatory power of regression (Wonnacott and Wonnacott 1981; Zar 1984).

Because fish length has been the only factor used for TS predictions to date, the relationship between the maximum cross-section of the swim-bladder and standard length was examined by means of correlation analysis. The software "Statgraphics-plus" was used for all statistical procedures. All of the statistical inferences were based on the 0.05 significance level.

Results

The ranges of the variables are shown in Table 1, and their seasonal variations are shown in Fig. 1. The specimens ranged from age 0+ to 95% of L_∞ (Tserpes and Tsimenides 1991). The summer 1989, summer 1990, and spring samples were from the nonreproductive period, whereas the autumn and winter samples were from the reproductive period. The summer 1989 sample included mainly small fish (age 0+), with low lipid content. All of the summer samples had a high degree of stomach fullness; the spring samples had varying degrees of fullness, and the samples collected during the reproductive period either had empty stomachs or contained very little food. The variation in the relative stomach volume is a consequence of the degree of fullness as well as fish thinness. The swim-bladder tilt angle relative to the horizontal plane had a mean of 6° with low variability (standard error ±0.02).

Cluster analysis

For the calculation of Mahalanobis distances we used the standard lengths in centimetres, and the relative lipid content, relative gonad volume, relative stomach volume, and swim-bladder volume in cubic centimetres. The results, using the average, centroid, and median cluster analysis methods, were similar. Cluster analysis (Fig. 2) showed two distinct groups: one for the reproductive period and one for the nonreproductive period. It is interesting that the summer 1990 sample was more closely related to the spring sample than to the summer 1989 sample.

Correlation analysis

In the samples collected during the nonreproductive period, there was a clear positive correlation between swim-bladder geometric cross-sectional area and fish length. There were no statistically significant correlations in the samples collected during the reproductive period (Table 2).

Cross-section size

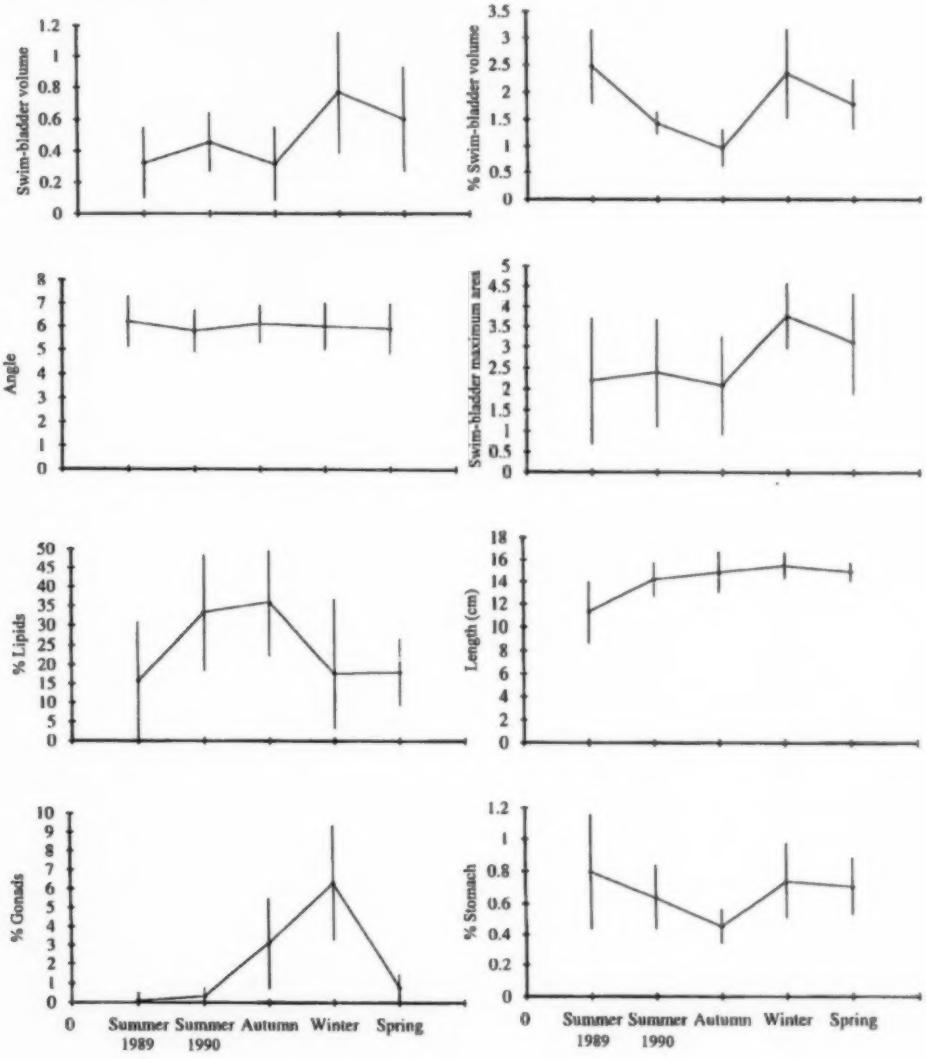
Multiple regression equations were estimated using the variables length, relative lipid content, relative gonad volume, and relative stomach volume (Table 3). Seasonal relationships were estimated from each sample and the interseasonal relationships from combined samples using cluster analysis. The annual relationship was estimated by combining all of the samples. For the variable length (L) the use of regressor log L, instead of a regressor L^b or L^2, resulted in models that fit the data better.

The sequence changes in the explained variance of each additional variable are shown by the successive R^2 values (Table 4). All of the regressions were linear. Lipid content was positively correlated with the maximum swim-bladder cross-sectional area in two cases (summer 1990 and spring) and negatively correlated in the others. Relative gonad and stomach volumes were positively correlated with cross-sectional area. Log L was positively correlated in the samples collected during the nonreproductive period (six cases) and negatively during the reproductive period (three cases).

In the nonreproductive period, the main variable (i.e., the variable explaining the highest proportion of variance) was length (regressor log L). The importance of this regressor was slightly reduced in the spring sample, where stomachs had varying degrees of fullness. In the reproductive period, the main regressor was relative gonad volume, whereas the importance of lipids was significant in the autumn sample. The regressor log L was introduced in the stepwise multiple regression only after the influence of the other variables had been removed; however, even then it explained only a very small proportion of the total variation.

The fit of the annual model was poorer than that of the other relationships. Without using the dummy variable D_g, the relationship had an $\overline{R}^2$ value of 0.56. To introduce the influence of the reproductive period, the dummy variable D_g was used. The regressor log L and the regressor D_g explained a greater percentage of the variation of the dependent variable. The dummy

Fig. 1. The interseasonal changes in variables, with 95% confidence limits. The swim-bladder volume is in cubic centimetres; swim-bladder maximum cross-sectional area is the dorsal swim-bladder maximum cross-sectional area in square centimetres; % swimbladder volume is the percentage of swim-bladder volume in terms of the wet weight of the fish (relative swim-bladder volume); angle is the tilt angle of the maximum cross-sectional area in relation to the initial reference plane in degrees; % lipids is the percentage of total lipid weight in terms of the dry weight of the fish (relative lipid content); length is the standard length of the fish in centimetres; % gonads is the percentage of gonad volume in terms of the wet weight of the fish (relative gonad volume); % stomach is the percentage of stomach volume in terms of the wet weight of the fish (relative stomach volume).



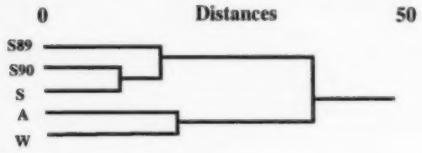
variable split the model into two parts, one for the reproductive period and one for the nonreproductive period.

Cross-section shape

The changes in circularity of the maximum swim-bladder cross-sectional area (Fig. 3) were examined by means of correlation analysis between C_s (circularity of the shape) and the fish's length, relative lipid content, relative gonad volume, and relative stomach volume (Table 5).

Because the ratio C_s tends to a minimum value (4π) as the shape of the cross-section approaches that of a circle, negative correlations indicate a decreasing value of the ratio C_s; that is, it approaches its minimum value, corresponding to a full circle. On the contrary, positive correlations indicate an increasing value of the ratio C_s, and hence a decrease in circularity.

Fig. 2. The result of cluster analysis by the average method. S89, summer 1989; S90, summer 1990; S, spring; A, autumn; W, winter.



The relative lipid content always had a positive correlation trend with cross-section. The correlations were statistically significant in the autumn, winter, and autumn–winter samples. The relative gonad volume always had a negative correlation, which was statistically significant only in the autumn–winter sample. Relative stomach volume was negatively correlated with C_s, but the correlation was never statistically significant. Length was always negatively correlated and these correlations were statistically significant in the summer 1989, summer 1990, spring, summer 1990 – spring, and autumn–winter samples.

Discussion

Because the swim-bladder is considered to be responsible for a major part of a fish's acoustic backscattering energy (Foote 1980), any change in the size of the swim-bladder geometric cross-sectional area affects fish TS. The swim-bladder of the sardine has a tilt angle of about 6° upwards relative to the horizontal plane, which agrees with estimates in other clupeids, e.g., 7.5° in herring (Blaxter and Batty 1990).

Cluster and regression analyses revealed the need for two distinct models for the calculation of cross-sectional area, one for the nonreproductive period and the other for the reproductive period. In the nonreproductive period, the main variable affecting cross-section is length (explaining 75–85% of the total variation). In this period, swim-bladder cross-sectional area increases as fish size increases. This agrees with the well-known positive correlation of TS with the logarithm of fish length (MacLennan and Simmonds 1992). However, during the reproductive period, the main variable is relative gonad volume (explaining 51–73% of the total variation). This implies a deviation from the aforementioned well-known correlation and supports Ona's (1990) statements on gadoids. During the reproductive period, lipid content made a significant contribution to explaining the variation in the cross-section (12–27%), and length decreased in importance (explaining only 2–20% of the total variation). During the reproductive period, the swim-bladder cross-sectional area increased in accordance with relative gonad size rather than fish size. Sex did not seem to have any particular influence on maximum swim-bladder cross-section.

The regressor length (log L) had a positive sign during the nonreproductive period (six cases) and a negative one during the reproductive period. During the reproductive period there were also no statistically significant correlations between cross-sectional area and fish length (Table 2). In addition, length (log L) was not the primary regressor; it was introduced into the relationships (as the second or the third regressor) only after accounting for the influence of gonads. As the gonad and secondary lipids explained the major part of the total variation, the effect of length was rather to improve the fit, and the negative sign may be a statistical artifact. There could be a biological explanation as well: as the fish increase to maximum size, the swim-bladder also approaches its upper size limit. In this case, it has been observed that the usual positive allometric relationship is replaced by isometric or small negative allometric relationships (Saenger 1989). The negative sign may also be due to the large size of the fish in the samples, especially in winter (the sample contained fish close to L_∞). These effects of length, combined with the increase in gonad size and the decrease in lipid content, imply that length should not be used for the calculation of the swim-bladder cross-sectional area during the reproductive period. Consequently, it can be surmised that during the reproductive period, gonad size affects TS calculations much more than length.

Table 2. Correlation analysis between the geometric maximum cross-sectional area of swim-bladder and length.

Sample	Correlation	
	r	p
Summer 1989	+0.921	0.000
Summer 1990	+0.913	0.000
Spring	+0.766	0.010
Autumn	+0.204	0.571
Winter	–0.459	0.182
Summer 1990 – spring	+0.865	0.000
Summers 1990 and 1989 – spring	+0.739	0.000
Autumn–winter	+0.236	0.316
Annual	+0.549	0.000

Note: r, correlation coefficient; p, significance level of the correlation.

The relative lipid content had a positive sign in two cases (summer 1990 and spring samples) and was negative in the others. Because lipid content and swim-bladder size are complementary factors insofar as buoyancy is concerned (primary lipid effect), a negative sign in the correlation between them is expected (Blaxter and Batty 1984; Ona 1990). This is the case for samples with a wide range of lipid values. In narrower ranges of lipid values, the positive sign could be a statistical artifact implying that the effect of lipid depends on specific combinations of length and lipid values. There could be a biological explanation here as well. The positive sign may imply a secondary effect of lipids: a dorso-ventral compression of the swim-bladder, resulting from the main dorso-ventral storage of the lipids (Hickling 1945). In these cases, fish with the same size of swim-bladder could have a slightly greater cross-sectional area because of the effect of lipids (a microscale effect of lipid). This effect also implies that changes in the swim-bladder volume are smaller in the presence of high lipid content (Iles and Wood 1965).

An interesting pattern was observed in the summer 1989 sample, which contained many small fish. In fish smaller than 11.5 cm, no lipid tissue was visible, probably because of the faster growth of young fish. This difference may explain why in cluster analysis the summer 1990 sample was more closely associated with the spring sample than the sample from summer 1989. This was also expressed by the lower value of $\overline{R^2}$, when the sample of summer 1989 was combined with the summer 1990 and spring samples.

Table 3. Seasonal and interseasonal multiple regression for the seasonal samples and their interseasonal combinations according to the cluster analysis.

Sample	Regression equation	R^2	n	SE
Summer 1989	A=16.2638(log L)+2.4288G–0.0566F–14.3404	0.920	10	0.247
Summer 1990	A=24.3995(log L)+0.0189F–26.3466	0.822	10	0.292
Spring	A=43.5538(log L)+3.2799S+0.0468F–51.1680	0.824	10	0.212
Autumn	A=0.3898G–0.0895F–13.2559(log L)+19.6230	0.969	10	0.111
Winter	A=0.2098G–18.6221(log L)+24.5979	0.691	10	0.249
Summer 1990 – spring	A=29.7997(log L)–31.8976	0.754	20	0.373
Summers 1989 and 1990 – spring	A=9.9821(log L)+1.4927S–9.7217	0.529	30	0.453
Autumn–winter	A=0.3325G–0.0274F–1.7809(log L)	0.854	20	0.381
Annual	A=7.8004(log L)–0.0151F+0.3351G–1.4385D_g–6.00	0.854	50	0.543

Note: $\overline{R^2}$, adjusted R^2; n, number of observations (specimens); SE, standard error of the estimate; A, maximum cross-sectional area in square centimetres; L, fish standard length in centimetres; F, relative lipid content; S, relative stomach volume; G, relative gonad volume; D_g, the dummy variable with values of 1 and 0.

Fig. 3. Characteristic maximum swim-bladder cross-sections, with the minimum circumscribed circle for each sample.

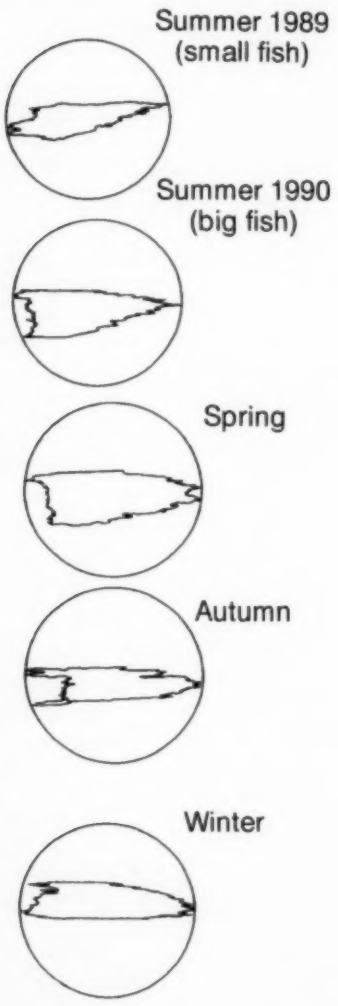


Table 4. The relative importance of each regressor.

Sample	Regressor	R^2	SE
Summer 1989	log L	0.849	3.779
	G	0.907	0.806
	F	0.947	0.023
Summer 1990	log L	0.819	4.096
	F	0.867	0.008
Spring	log L	0.669	3.111
	S	0.885	0.403
	F	0.981	0.008
Autumn	G	0.612	0.043
	F	0.882	0.012
	log L	0.981	2.899
Winter	G	0.515	0.054
	log L	0.744	5.142
Summer 1990 – spring	log L	0.754	4.628
Summers 1989 and 1990 – spring	log L	0.494	1.726
	S	0.563	0.732
Autumn–winter	G	0.731	0.044
	F	0.854	0.007
	log L	0.871	0.838
Annual	log L	0.329	1.656
	F	0.445	0.007
	G	0.506	0.061
	D_g	0.669	0.312

Note: The sample column gives the sample or combination of samples used. The regressors column gives the regressors used in the respective regressions of Table 3, in order of importance (i.e., decreasing contribution to maximization of R^2). In the R^2 values column each value indicates changes in R^2 attributed to each additional regressor. The standard error values are those of the coefficient of each successive regressor. L, fish standard length; F, relative lipid content; S, relative stomach volume; G, relative gonad volume; D_g, the dummy variable with values of 1 and 0.

The stomach seems to have a marginal or no influence on the maximum swim-bladder geometric cross-sectional area. This result differs from Ona's (1990) conclusions for gadoids. According to Ona, "in fish with full stomachs, the swim-bladder is deformed as to be almost unrecognizable." This difference could be because of the distinct fish type examined in each case. Specifically, gadoids are physoclistous carnivores, feeding on large compact organisms. Consequently, stomachs should be able to enlarge greatly depending on the

Table 5. Correlation between C_s (circularity of swim-bladder shape) and the independent variables.

	Lipid		Gonad		Stomach		Length	
Sample	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Summer 1989	–0.499	0.142	–0.553	0.102	–0.133	0.714	–0.667	0.043
Summer 1990	–0.052	0.887	–0.360	0.307	–0.342	0.307	–0.578	0.083
Spring	+0.171	0.636	–0.503	0.138	–0.411	0.237	–0.734	0.016
Autumn	+0.776	0.008	–0.366	0.298	–0.006	0.855	–0.561	0.091
Winter	+0.620	0.053	–0.361	0.306	–0.369	0.293	+0.537	0.110
Summer 1990 – spring	+0.173	0.467	–0.322	0.166	–0.325	0.163	–0.686	0.001
Summers 1990 and 1989 – spring	+0.178	0.348	–0.194	0.305	–0.234	0.214	–0.097	0.609
Autumn–winter	+0.646	0.002	–0.491	0.027	–0.441	0.052	–0.517	0.020
Annual	+0.175	0.225	–0.150	0.299	–0.164	0.254	–0.154	0.286

Note: The length column gives the fish standard length in centimetres; the lipid column gives the relative lipid content; the stomach column gives the relative stomach volume; the gonad column gives the relative gonad volume. *r*, correlation coefficient; *p*, significance level of the correlation.

size of prey, which evidently has a great effect on the swim-bladder. Sardines, however, are physostomous, filter-feeding planktivores, which may not require the stomach volume to undergo great changes (Hickling 1945). In view of this, the effect of stomach fullness on maximum swim-bladder cross-section is therefore limited.

From the results of the shape study, four preliminary conclusions may be drawn. (*i*) The swim-bladder tends to become more circular as the fish length increases. This may indicate an allometric change in the swim-bladder with fish size. (*ii*) The swim-bladder tends to become more circular as the relative gonad volume increases. As gonads grow, there is a gradual compression of the posterior part of the swim-bladder, which increases the circularity, especially during the reproductive period (see the autumn–winter sample in Table 5). This pattern is similar to the conclusions of Ona's (1990) study on gadoids, where a dorso-ventral deformation of the posterior of the swim-bladder was also observed in the late maturity stages. (*iii*) The swim-bladder tends to become less circular with increasing lipid content, probably reflecting the dorso-ventral pattern of storage of lipids (Hickling 1945). This type of storage may be the cause of irregularities in swim-bladder cross-sectional shape, which increases the perimeter. This result supports the hypothesis of a secondary lipid effect. (*iv*) The stomach seems to have a marginal or no effect on swim-bladder geometric maximum cross-sectional shape.

To summarize, the swim-bladder geometric cross-sectional area can be calculated from two distinct models. However, a third model might be needed for young small fish because of their lower lipid content. According to our results, physiological factors seem to be very important in the computation of the TS. Small fish with little or no lipid had swim-bladder cross-sectional areas almost the same as those of larger fish with a high lipid content. Fish with high relative gonad volumes and low lipid contents had greater swim-bladder cross-sections (and presumably greater TS) than those calculated from fish size alone (Fig. 1). This may lead to either underestimation or overestimation of fish size from acoustic backscattering and consequently to overestimation or underestimation of fish abundance.

Ona (1990) pointed out that the bulk of experimental data on TS is based on immature fish, mostly gadoids (in which length explains a much higher proportion of swim-bladder variability). As for fishes of colder regions where the reproductive period is short, length can probably efficiently predict TS for most months of the year. However, in subtropical conditions, such as those that prevail in the Greek seas, where the reproductive period is much longer (for sardine it lasts almost half a year), the relationship of gonads to the swim-bladder is as important as that of the swim-bladder to fish length.

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References

Blaxter, J.H.S., and Batty, R. 1984. The herring swimbladder: loss and gain of gas. J. Mar. Biol. Assoc. U.K. **64**: 441–459.

Blaxter, J.H.S., and Batty, R. 1990. Swimbladder "behaviour" and target strength. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, **189**: 233–244.

Buerkle, U. 1987. Estimation of the fish length from acoustic target strengths. Can. J. Fish. Aquat. Sci. **44**: 1782–1785.

Clay, C.S., and Medwin, H. 1977. Acoustical oceanography: principles and applications. John Wiley and Sons Inc., New York.

Culley, M. 1970. The pilchard. Biology and exploitation. Pergamon Press, Oxford.

Culling, C.F.A., Allisson, R.T., and Barr, W.T. 1985. Cellular pathology techniques. Part VIII. Quantitative methods. Butterwoth and Co. Ltd., London.

Farrant, J., Walter, C.A., Lee, H., Morris, G.J., and Clarke, K.J. 1977. Structural and functional aspects of biological freezing techniques. J. Microsc. (Oxford), **111**: 17–34.

Foote, K.G. 1980. Importance of the swimbladder in acoustic scattering by fish: a comparison of gadoid and mackerel target strengths. J. Acoust. Soc. Am. **67**: 2084–2089.

Foote, K.G. 1985. Rahter-hight-frequency sound scattering by swimbladdered fish. J. Acoust. Soc. Am. **78**: 688–700.

Foote, K.G., and Ona, E. 1985. Swimbladder cross-sections and acoustic target strengths of 13 pollack and 2 saithe. Fiskeridir. Skr. Ser. Havunders. **18**: 1–57.

Henderson, R.J., and Almatar, S.M. 1989. Seasonal changes in the lipid composition of herring (*Clupea harengus*) in relation to gonad maturation. J. Mar. Biol. Assoc. U.K. **69**: 323–334.

Hickling, C.F. 1945. The seasonal cycle in the Cornish pilchard, *Sardina pilchardus* (Walbaum). J. Mar. Biol. Assoc. U.K. **26**: 115–138.

Iles, T.D., and Wood, R.J. 1965. The fat/water relationship in North Sea herring (*Clupea harengus*) and its possible significance. J. Mar. Biol. Assoc. U.K. **45**: 353–366.

Love, R. 1969. Maximum side-aspect target strength of an individual fish. J. Acoust. Soc. Am. **46**: 746–752.

Love, R. 1977. Target strength of an individual fish at any aspect. J. Acoust. Soc. Am. **62**: 1397– 1403.

MacLennan, D.N., and Simmonds, E.J. 1992. Fisheries acoustics. Chapman and Hall, London.

Muzinic, R. 1970. On the use of anaesthetics in the transportation of sardines. Studies and Reviews. General Fisheries Council for the Mediterranean. F.A.O. **47**: 1–23.

Ona, E. 1990. Physiological factors causing natural variations in acoustic target strength of fish. J. Mar. Biol. Assoc. U.K. **70**: 107–127.

Saenger, R.A. 1989. Bivariate normal swimbladder size allometry models and allometric exponents for 38 mesopelagic swimbladdered fish species commonly found in the North Sargasso Sea. Can. J. Fish. Aquat. Sci. **46**: 1986–2002.

Squire, J.L. 1978. Northern anchovy shapes as related to problems in school size estimation. Fish. Bull. **76**: 443–448.

Tserpes, G., and Tsimenides, N. 1991. Evaluation of growth rate differences in populations of *Sardina pilchardus* (Walbaum, 1792) (Clupeidae) from the Aegean and Ionian Seas. Cybium, **15**: 15–22.

Verheijen, F.J. 1955. On a method for collecting and keeping clupeids for experimental purposes, together with some remarks on fishery with light-sources and a short description of free cupulae of the lateral line organ on the trunk of the sardine *Clupea pilchardus*. Mar. Ecol. **28**: 225–240.

Wonnacott, T.H., and Wonnacott, R.J. 1981. Regression: a second course in statistics. John Wiley and Sons, New York.

Zar, J.H. 1984. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Quantifying the impact of trawling on benthic habitat structure using high resolution acoustics and chaos theory[1]

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Abstract: Very high resolution and broadband parametric array acoustics were used to estimate the small-scale structural properties of surficial sediments as part of a trawling impact experiment on the sandy sediment of the eastern Grand Banks. The seabed was ensonified by a 12 × 30 cm, 40-element acoustic array (DRUMS™) deployed on the frame of a 0.5-m^2 bottom grab. Acoustic images of the upper 4.5 cm of sediment were taken in 10 sampling blocks along each of two corridors that were 13 km long, before and after intensive otter trawling. The acoustic return signals were Hilbert transformed and divided into five depth strata of 50 μs, or approximately 1 cm, from slightly above the average sediment surface to approximately 4.5 cm depth. The fractal of the transformed signal from each acoustic element was calculated for each depth stratum. The fractals of the acoustic returns from pretrawled sediments are consistently and significantly higher than those from trawled sediments in all five depth strata in both corridors. A chaos model, using fractals of the parametric array acoustic signals as metrics, provides an analytical framework in which the structural effects of physical disturbance of the benthic habitat can be quantified.

Résumé : Un réseau d'éléments acoustiques paramétriques à très haute résolution et à large bande a été utilisé pour estimer les propriétés structurales à petite échelle de sédiments superficiels dans le cadre d'une expérience portant sur l'impact du chalutage sur le sédiment sableux des Grands Bancs de l'Est. Le fond océanique a été balayé au moyen d'un réseau de 40 éléments acoustiques de 12 par 30 cm (DRUMS^{mc}) déployé sur le cadre d'une benne de fond de 0,5 m^2. Des images acoustiques des 4,5 cm supérieurs du sédiment ont été prises dans 10 blocs d'échantillonnage suivant deux corridors de 13 km de longueur, avant et après l'utilisation intensive d'un chalut à panneaux. Les signaux acoustiques de retour ont subi une transformation de Hilbert et ont été divisés en cinq couches de profondeur de 50 μs, soit approximativement 1 cm, entre un point situé légèrement au-dessus de la surface moyenne du sédiment et une profondeur d'environ 4,5 cm. La fractale du signal transformé de chaque élément acoustique a été calculé pour chaque couche de profondeur. Les fractales des signaux acoustiques de retour obtenues pour les sédiments avant le passage du chalut sont obtenues pour les sédiments après le passage du chalut pour les cinq couches de profondeur des deux corridors. Un modèle de chaos, dans lequel on utilise comme mesures les fractales des signaux des éléments acoustiques paramétriques, fournit un cadre analytique dans lequel les effets structuraux de perturbations physiques de l'habitat benthique peuvent être quantifiés.
[Traduit par la Rédaction]

Introduction

Physical disturbances of natural or human origin disrupt the structure of the soft bottom benthic habitat via direct contact or turbulent resuspension of surficial sediments. A common cause of physical disturbance in the Canadian Atlantic continental shelf seabed is the widespread use of mobile fishing gear (Messieh et al. 1991). Several types of mobile gear that come in contact with the bottom during fishing have been used over large areas of the continental shelf for the past half century. Of these, the most frequently and widely used is the otter trawl, the major gear type of the offshore groundfish fishery. An in situ experiment to determine the impacts of otter trawls on the sandy seabed, which covers large areas of the Grand Banks and Scotian Shelf, has been underway since 1993. It was initiated as part of the Northern Cod Science and Atlantic Fisheries Adjustment Programs (Rowell et al. 1996) in response to debate regarding the role of fishing technology in the collapse of the northern cod stock. The experiment is designed to quantify immediate impacts of trawling and to follow the time course of recovery of trawled sediments. We report here on observations made immediately after trawling in July 1994.

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Broad frequency spectrum and narrow beam acoustics (DRUMS™, Dynamically Responding Underwater Matrix Sonar, Guigné et al. 1993) have been developed by Guigné International Limited under contract to the Department of Fisheries and Oceans for high resolution measurement of structural changes in the benthic habitat caused by otter trawling. In situ, nondestructive imaging of macrofaunal structures such as tubes, burrows, and mollusc shells, within broad frequency spectrum signals, was demonstrated by Guigné et al. (1993).

The surfaces of many natural structures, including marine sediments, cannot be adequately characterized by measurement at a single scale of resolution. Fractal geometry (Mandelbrot 1982) has found wide use in numerical characterization and analysis of such structures. Microcrystalline solids, powders, porous solids, sediment aggregates, macroalgal fronds, coral reefs, and landscape patterns, for example, have all been described in terms of their fractal dimensions (e.g., Bradbury and Reichelt 1983; Gee and Warwick 1994; Jaggi et al. 1993; Kaye 1989; Kranenburg 1994; Lu and Hellawell 1994; Meyer et al. 1994; Wilson 1994). Applying the fractal model to marine sediments is appropriate, since the measured surface area of sediments is scale dependent within defined scales of resolution and generally increases with increasing resolution, both characteristics of fractal structures.

To allow quantitative comparisons of acoustic microstructure of sediments among sites in our trawling experiment, we cast DRUMS acoustic return signals from the sediments (time-history series) in the form of fractals. Our approach has been to postprocess Hilbert-transformed acoustic signals directly to fractal form, rather than to first interpret the acoustic structure of the sediment and then analyse micro-"landscape" patterns in terms of their fractal geometry. In this way, the analysis presented here is a departure from previous work in ecology and geology. The value of fractal geometry, applied to high resolution acoustic data, is demonstrated for quantitative comparison of undisturbed sediments with those whose natural texture and structure have been altered by external physical forces imparted by otter trawling. We introduce a nondestructive, quantitative technique for imaging the structure of the subbottom benthos that should be a useful tool for assessment of the effects of human activities on the seabed environment.

Site description

DRUMS acoustic data were collected during a trawling experiment in a 10-M (1 M = 1852 m) square, centred at 47°10′N, 48°17′W, approximately 60 km northeast of the Hibernia oil field on the eastern Grand Banks of Newfoundland. The average water depth in the area is 137 m. The currents in the area are weak except during the storms that frequent the area. Iceberg scours are common bottom features (Lewis and Woodworth-Lynas 1990). The bottom sediment is well-sorted sand with a modal grain size of approximately 200 μm and water content of approximately 18%. The particulate organic carbon content is in the range of 0.04–0.14%, with a mean of 0.08%.

The benthic macrofaunal community is abundant and diverse, with an average of over 2000 individuals/m^2 comprising an estimated 139 species (Rowell et al. 1996). An abundant epifaunal community is dominated by sand dollars (*Echinarachnius parma*), brittle stars (*Ophiura sarsi*), basket stars (*Gorgonocephalus arcticus*), and snow crabs (*Chionoecetes opilio*). Until the early 1980s, a mixed-species otter trawl fishery, dominated by American plaice (*Hippoglossoides platessoides*), was pursued in the area. It has not been heavily fished since that time, possibly because of the presence of an abandoned petroleum exploration well near the centre of the experimental area. The groundfishery has been closed by a moratorium that commenced in 1992, and the experimental area has been closed to all fishing for the duration of the trawling experiment, starting in 1993. Thus, there is little likelihood that experimental trawling results will be confounded by commercial trawling in the area.

Methods

Experimental design and sampling

We briefly outline relevant aspects of the experimental design and sampling procedures here; the details are given by Rowell et al. (1996). Three corridors 13 km long by 200 m wide (A, B, and C), laid out in a triangular pattern, were charted in the experimental area. Parallel control (untrawled) areas were laid out for biological sampling. Each corridor was trawled 12 consecutive times by the RV *Wilfred Templeman* using an Engels 145 otter trawl equipped with rock-hopper foot gear similar to the otter trawls used by commercial draggers. A Scanmar gear mensuration system on the *Templeman* indicated that the width of the trawl, between the otter boards, was a constant 60 m, and the mouth of the trawl was a constant 16 m across. Each corridor took 30–35 h to complete, trawling at 3.5 kn (1 kn = 1.852 km/h), and all of the trawling for the work described here took place between July 8 and July 16, 1994. Using differential GPS navigation, the ship maintained course with a maximum of 20 m cross-track error; most of the time the ship was within 5–10 m of the nominal centre line of the corridor. The zone of trawling disturbance was 100–140 m wide, between otter boards.

The CSS *Parizeau* sampled corridors A and B immediately before and after each had been trawled. Corridor C could not be sampled by the *Parizeau* because of time constraints. The corridors were charted with 260–50 m long sampling blocks. Ten randomly preassigned sampling blocks along the lengths of corridors A and B were sampled before and after trawling. Samples were taken near the center of the sampling blocks where trawling definitely had occurred. A video-equipped, 0.5-m^2 hydraulic grab (Rowell et al. 1994) on which the DRUMS was placed (Fig. 1) was one of the primary sampling devices. The frame of the grab was lowered to rest on the sediment surface prior to sampling. The jaws were then operated hydraulically from surface electronic controls. After the grab was taken and the jaws were completely closed, the entire apparatus was raised. If the jaws did not close completely, the upper grab covers were seen on the video not to have closed fully, and the grab could be recocked to take another sample without bringing it to the surface. The grab was lowered very gently using video guidance and was driven into the sediment hydraulically rather than by force of impact, thereby minimizing surface disturbance. High resolution video and DRUMS acoustic imaging were completed before the grab was closed and brought to the surface for biological and sediment sampling. The video images indicated that the sediment surface was virtually undisturbed by the grab impact.

The seabed was ensonified at each grab sample a few seconds after the grab frame came to rest on the sediment surface. The Benthic-DRUMS was configured as a modular 4 × 10 array of transmitter–receivers placed 2.8 cm centre to centre (Fig. 2). The array was mounted in the grab frame, above the open grab jaws, and approximately 1.3 m above the sediment surface (Fig. 1). The area of sediment ensonified was approximately 12 × 30 cm, extending

Fig. 1. Video-equipped, 0.5-m^2 hydraulic grab with DRUMS. An electronic bottle (1) containing the programmable wave shaping and feedback controllers, amplifiers, and data acquisition equipment is secured onto the main frame (2) away from the sampler jaws. The video camera is noted at 3.

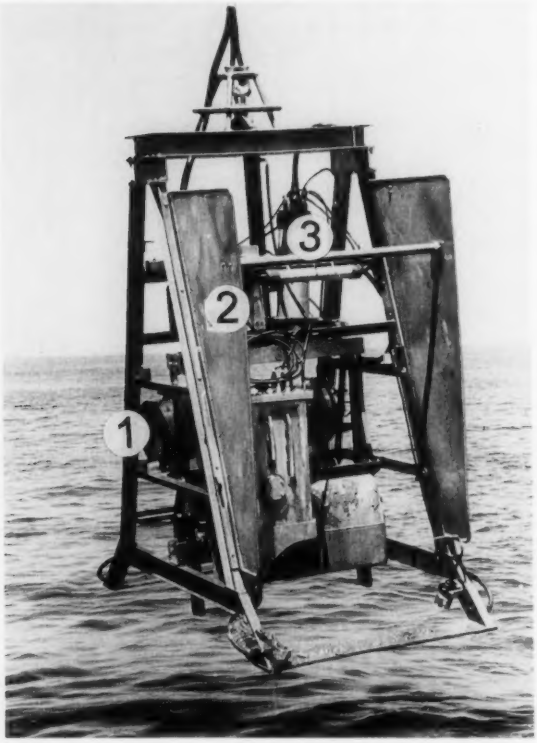


Fig. 2. DRUMS sonar head (1) with its 40 transmitters and receivers placed above the grab sampler's hydraulic guide frame (2), 1.3 m above the base.

from near the centre of the grab to near one side edge, along the hinge axis of the grab. Preliminary tests in an equipment test tank at the Bedford Institute of Oceanography assured us that the matrix of acoustic "pencil beams" emitted by DRUMS minimized acoustic interference from reverberations within the grab. The 40 elements of the array were triggered sequentially, taking a total of less than 1 min for a complete digital DRUMS image to be completed and stored. The entire acoustic imaging procedure required less than 5 min added to the grab sampling time. One station in corridor A, before trawling, was not imaged by DRUMS because the power was interrupted before the data were stored.

DRUMS

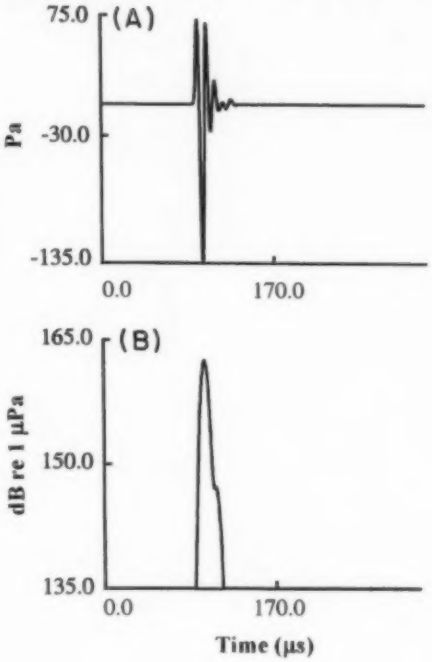
Guigné et al. (1993) describe the importance of using very high resolution and broad frequency spectrum acoustics for benthic ecological studies. DRUMS employs nonlinear acoustics to achieve the required depth of penetration, high temporal resolution, and lack of reverberation interference. Sediment features of strong impedance contrast, including heavy-shelled fauna such as some bivalve molluscs, are resolvable as separate energy peaks; much smaller scale sediment properties and other fauna produce a redistribution of the energy in the form of signal attenuation and of low energy reflective returns. The system utilizes the principle that the change in density caused by a change of pressure of a sound wave in water is not linearly proportional to the change in pressure. For sinusoidal acoustic waves in water, a variety of additional frequencies are generated (Urick 1975). If a signal is transmitted at high enough power, the generated acoustic wave will propagate in the water in a distorted manner; the nonlinearity causes a dependence of the propagation velocity on the pressure amplitude of the sound wave. The result is the creation of harmonics at the expense of the fundamental frequency (Urick 1975). This nonlinearity in water also causes the sum and difference frequencies to be generated when two or more wave sinusoids are propagated. Nonlinear interaction of sinusoids was first described by Westervelt in 1963, and an extensive literature now exists on the physics (refer to Berktay and Leahy 1974; Moffet and Mellen 1977; Beye 1965; Mellen and Moffett 1978; Willette and Moffett 1977 for introductory references). Characteristics of the nonlinear acoustic propagation by DRUMS can be summarized as follows: (*i*) narrow beam spread and no side lobes, (*ii*) low propagation frequencies, (*iii*) wide bandwidth, (*iv*) small radiator size, and (*v*) capability of operation at a variable range of frequencies and power levels.

Each of the 40 transmitting and receiving elements of the DRUMS consists of a modular nonlinear sonar. The sonar head (Fig. 2) houses the 40-element array along with control hardware (decoder, relays, preamplifiers, multiplexer). The DRUMS controllers, amplifier, signal conditioning waveform generators, and a 486 PC computer board and its controllers were housed in a pressure-tested electronics cylinder mounted on the video grab frame (Fig. 1). A shipboard 486 PC computer was used to perform further signal processing and time series analysis.

The acoustic data were Hilbert transformed to enhance the display of the returned signals. Guigné et al. (1991) and Guigné and Chin

(1989) provide details on the application of the Hilbert transform to underwater acoustics. The analytic signal created by the transform contains the imaginary part of the signal along with the real part. Signals are displayed as time or frequency envelopes. The transformed data allow more precise time representation of both the peak values and instantaneous phases of the signal. The Hilbert-transformed series were detrended and the energy envelopes were log transformed. An example of the outgoing waveform from the DRUMS along with its Hilbert-transformed envelope is shown in Fig. 3. In addition, the Hilbert-transformed time histories for one row of 10 elements from the sonar array are included (Fig. 4) as an illustration of the raw unfiltered data that were collected.

Fig. 3. An example of the outgoing acoustic waveform generated by DRUMS, with its Hilbert transform envelope. The amplitude of the real part of the signal (A) is in pascals. The magnitude of the pseudo-energy envelope of the Hilbert transform (B) is in decibels relative to 1 μPa, permitting the peaks to be presented on a log scale. The relation to 1 μPa is the standard reference used in underwater acoustics.



Fractal analysis

The Hilbert-transformed acoustic signals occupy 1-dimensional topological space; however, they are more complex than a simple straight line, and they fill 2-dimensional space to a degree that depends on the frequencies and amplitudes of the peaks and undulations that make them complex. The extent to which they fill 2-dimensional space is measured by a noninteger dimension between 1 and 2, called a fractal. By analysing the time series data as fractal curves, the complexity of the acoustic signals in response to structural complexity in the sediment can be quantified. Drawing the connection between the signal fractal and sediment structure requires that the signal-to-reverberation ratio in the data be extremely high, a consequence of the broad bandwidth and narrow beam spread of the acoustics. The scale at which DRUMS functions produces very exact signals with no reverberation interference and devoid of side lobes, as required to resolve benthic structures coherently.

Fractal values were calculated in five depth strata, each 50 μs or approximately 1 cm thick. The first stratum included the average sediment surface, to a depth of approximately 0.5 cm. The second stratum extended from 0.5 cm below the average sediment surface to a depth of approximately 1.5 cm. The bottom of stratum 5 extended to approximately 4.5 cm depth in the sediment.

A modified box counting method (see Malinverno 1990) was used to determine the fractal for the Hilbert time histories from each depth stratum k of each acoustic element M_{ij} of the acoustic array. In this method, the data M_{ijk} were first fully enclosed in a square; thus, one box contained the whole line. Then the box was divided into quarters and the number of these smaller boxes that contained parts of the line was counted. Each of these smaller boxes was then similarly divided and the number containing parts of the line counted, and so on until arbitrarily small box size.

The number of boxes, N, containing the line is a function, f, of the size, r, of the boxes:

$$N = r^{-f}$$

The function, f_{ijk}, is the fractal dimension for each depth stratum of each element. It is calculated as

$$f_{ijk} = \frac{\mathrm{Log}_{10} N}{\mathrm{Log}_{10}(1/r)}$$

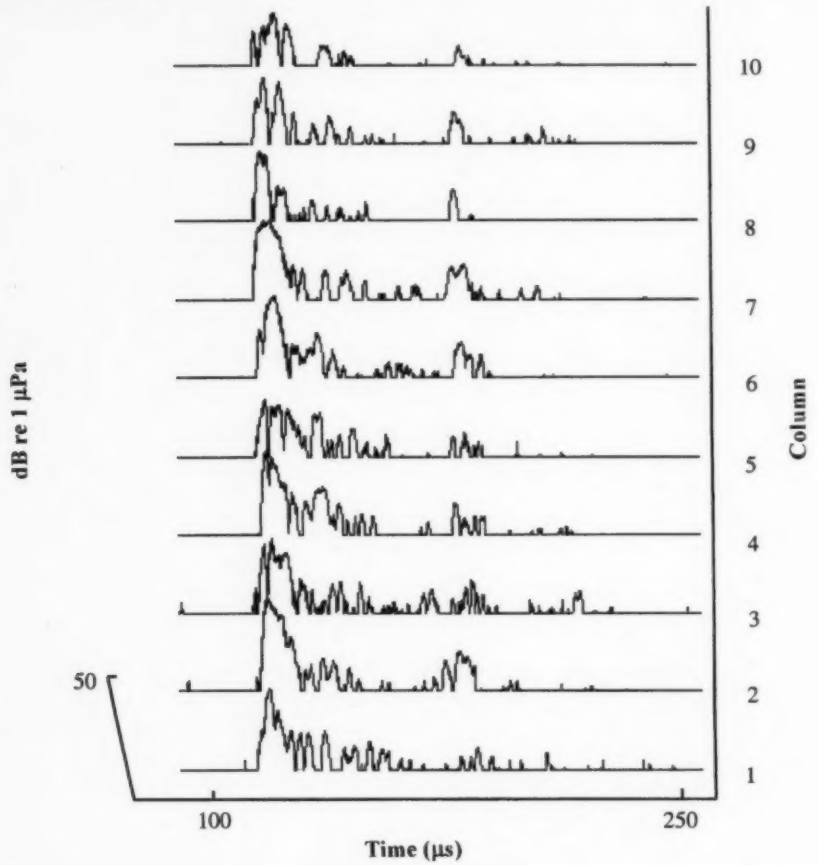
To produce the examples of interpolated fractal matrices plotted in Fig. 5, the 20 × 10 matrix of fractals for each acoustic array (4 rows of elements, 5 depth strata × 10 columns of elements) was cubic spline interpolated to produce a 155 × 121 matrix. The interpolated fractal values illustrated range between 1.1 and 1.6.

Statistical analysis

The plots of fractals over the areas covered by DRUMS provide a visual impression of the differences in sediment acoustic structure before and after trawling, and among samples, over five depth strata. To assess the significance of these differences within each depth stratum, an analysis of variance (ANOVA) was performed on the uninterpolated fractals. A Monte Carlo test of correlations of fractals among acoustic elements within and among arrays indicated that spatial correlations within arrays were not significantly stronger than chance correlations among arrays. Therefore, we considered that each element provided an independent acoustic measurement of sediment structure. One uncalibrated element produced consistently outlier data, which were eliminated from any analysis. For the purpose of ANOVA, the samples were not considered to be paired for two reasons: variances were high among replicate faunal grab samples taken in a preexperimental survey of the experimental area (Jens Prena and Terry Rowell, Bedford Institute of Oceanography, unpublished data), and the distances between before and after trawling samples within the sampling blocks were in the range of 10–50 m, so samples within a sampling block may not have been taken from the same faunal patch. Hence, for our purposes, it would be a mistake to consider the before and after trawling samples as paired.

For each depth stratum, the array thus provided 390 acoustic measurements over the 10 sampling blocks along each corridor, before and after trawling (351 along corridor A before trawling because of missed sample). The analysis of variance for each depth stratum is a nested design with two levels in each of the two treatments (corridor A and B, and before and after trawling), and the trawling treatment nested within the corridor "treatment" (Tables 1 and 2). In addition to univariate ANOVAs for each depth stratum, a MANOVA was performed to determine the significance of the experimental treatments over all five strata (Table 3). In this way, the experiment-wise error in what was essentially a multivariate experiment could be assessed (mixed decision strategy, Barker and Barker 1984). The Minitab for Windows procedure GLM, ANOVA with MANOVA (Minitab Inc. 1993), was used to perform this analysis.

Fig. 4. Hilbert-transformed acoustic return signals (time histories) for one row of 10 elements from the sonar array.



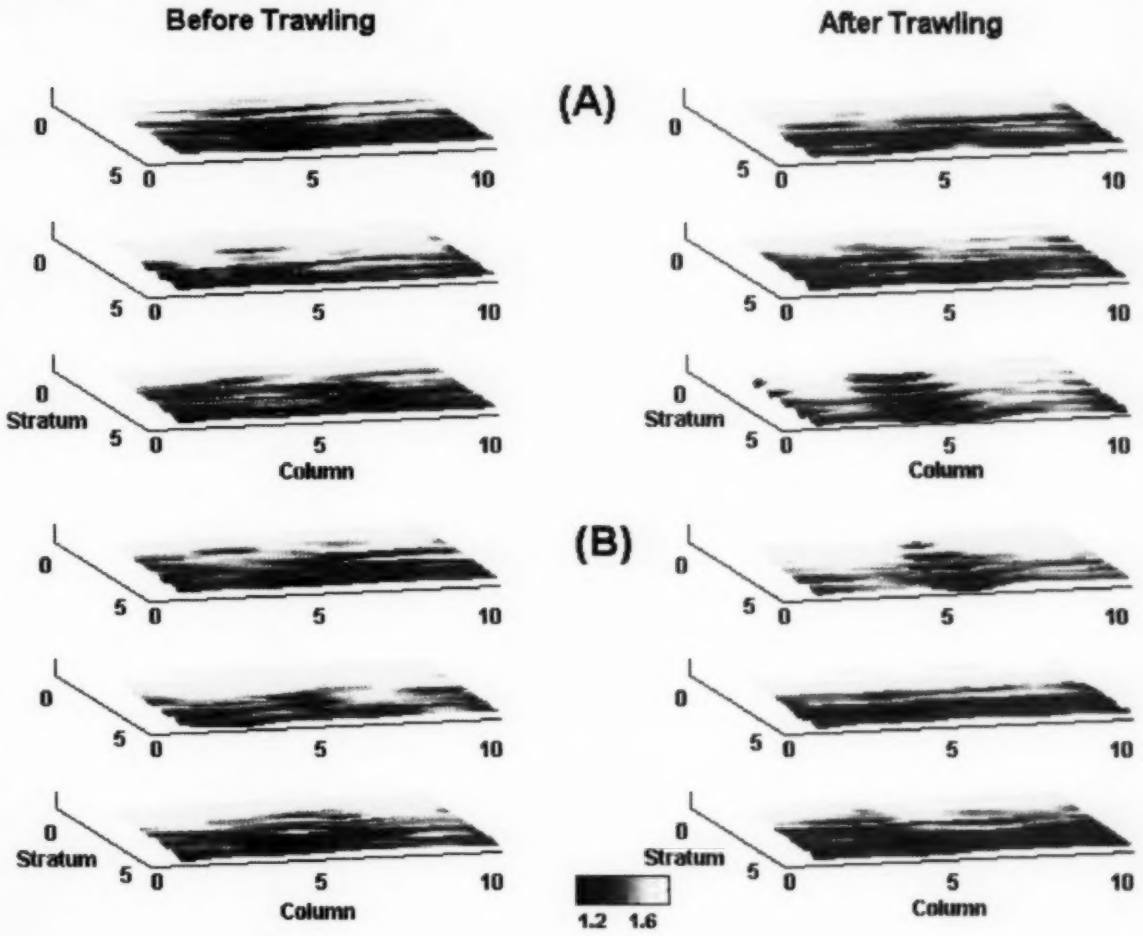
Results

The parametric array acoustics used in DRUMS provided exceptionally high temporal and spatial resolution. Examples of Hilbert-transformed, returned time histories for trawled and untrawled sites are given in Fig. 6. From these plots it is clear that rich structure was resolved in the upper 4.5 cm of the seabed sediment. The use of the Hilbert transform on the wavelets extended the resolving processing capacity of the approach, structuring the signals into pseudo-energy time curves that provided coherent inputs to the fractal processing. The Hilbert-transformed envelope histories provided for sharp boundary delineations of their near-surface internal features. In the case of plot B (trawled), diminished echo strengths existed in the near-surface subseabed compared with plot A (untrawled), which demonstrated multiple reflective energy returns from within the subseabed. Plot C, which was taken from an untrawled site, also contrasted sharply with plots B and D. In relation to plot A, a similar pattern is to be noted whereby high peaked histories are characteristic of untrawled habitats. The sharper energy peaks of A and C suggest more distinct or contrasting boundaries for the acoustics to reflect from. Higher energy envelope strengths were also noted with depth in undisturbed seabeds than were observed in the lower subseabed regions after trawling. These differences in structural subtleties between the trawled and nontrawled sites occurred consistently in all of the data. Results of an ANOVA (Tables 1 and 2), contrasting fractal values before and after trawling, indicated that the differences were highly significant.

The high resolution of DRUMS, resulting from its broad acoustic spectrum (30–300 kHz) and high coherence, is unattainable using conventional acoustic sources. Since side lobes and widebeam footprints are not generated, the echo returns can be taken as true indications of the physical influences found in the sediment structure. This is a critical consideration if one is to assign quantitative meaning to fractals.

Owing to sample collection requirements of other participants in the trawling impact project, it was not possible to associate the acoustic structure directly with subsurface structures in the sediment. Some general relationships are apparent, however. The high resolution video images of the 0.5-m^2 sediment surfaces before and after trawling (T.W. Rowell, Bedford Institute of Oceanography, unpublished data) generally showed that one impact of the trawling was to reduce the surface roughness of the seabed. Ripples, detrital aggregations, and surface traces of bioturbation were smoothed over

Fig. 5. Cubic spline interpolated fractal matrices of the 4 × 10 element array for five depth strata in three sampling locations before and after trawling (A) in corridor A and (B) in corridor B. Column is the interpolated position along the 10-element axis of the acoustic array, and the width of each depth stratum illustrated consists of the interpolated fractals on the 4-element axis. The interpolated fractal values range between 1.1 and 1.6. Fractals around 1.45 are red. Lower fractal values are darker (black to dark red) than higher ones (orange to yellow to white).



by the suspension and resettling of the surface sediment. The DRUMS acoustic data also indicated this alteration of surface structure, but the effect appeared to penetrate to at least 4.5 cm depth in the sediment (Fig. 6). The number and magnitudes of peaks were reduced in the histories of the returning energy after trawling. The interpolated fractal data for selected samples in Fig. 5 illustrate the same general pattern. Especially in the upper strata, but also to some extent in the deeper ones, the lighter colours associated with higher fractal acoustic time series were more dominant before than they were after trawling. The plots illustrate a uniformly rougher structure in the surface sediments before trawling than after trawling. Although the colour structure of the plots depicting structure after trawling appears to be more heterogeneous, the darker colour indicates that there was a general, though uneven reduction in the complexity of the internal sediment structure.

Differences in the fractals of the Hilbert-transformed time series before and after trawling, within each corridor, were statistically highly significant, but the differences between the two corridors were not consistently significant. The results of ANOVA in Tables 1 and 2 indicate that the reduction of chaotic structure after trawling extended to the deepest stratum analysed. With the exception of the surface of corridor B (stratum 1), fractals of all depth strata were consistently less after trawling than before. All of the parameters calculated in the MANOVA indicate very highly significant multivariate treatment effects. The mixed decision strategy recommended by Barker and Barker (1984) allows the high significance of the ANOVAs for all depth strata to stand.

Discussion

The ecological consequences of trawling on the benthos result from the manifold physical effects of trawling, one of which

Table 1. Analysis of variance for effect of corridor (A or B) and trawling on the fractal value of high-resolution acoustic data from five depth strata.

	df	SS	MS	*F*	*P*
Stratum 1					
Corridor	1	0.014 270	0.014 270	5.29	0.022
Trawl (corridor)	2	0.062 046	0.031 023	11.51	0.000
Error	1517	4.089 850	0.002 696		
Total	1520	4.168 435			
Stratum 2					
Corridor	1	0.000 027	0.000 027	0.01	0.922
Trawl (corridor)	2	0.033 158	0.016 579	5.80	0.003
Error	1517	4.335 913	0.002 858		
Total	1520	4.369 148			
Stratum 3					
Corridor	1	0.028 963	0.028 963	6.76	0.009
Trawl (corridor)	2	0.052 253	0.026 126	6.10	0.002
Error	1517	6.497 509	0.004 283		
Total	1520	6.577 235			
Stratum 4					
Corridor	1	0.007 156	0.007 156	1.40	0.238
Trawl (corridor)	2	0.222 951	0.111 475	21.73	0.000
Error	1517	7.781 110	0.005 129		
Total	1520	8.010 212			
Stratum 5					
Corridor	1	0.001 266	0.001 266	0.24	0.627
Trawl (corridor)	2	0.132 549	0.066 274	12.37	0.000
Error	1517	8.125 954	0.005 357		
Total	1520	8.260 818			

Note: The ANOVA design was GLM, nested ANOVA with MANOVA. The design takes into account one malfunctioning element in the array and one missed sample in corridor A before trawling. For treatment "corridor," there were two levels with values A and B. For treatment "trawl" (within corridor), there were two levels with values 1 (before) and 2 (after). In terms of response variables, fractal values were determined for five depth strata: 0–0.5, 0.5–1.5, 1.5–2.5, 2.5–3.5, and 3.5–4.5 cm. For each depth stratum, there were 351 replicates in corridor A before trawling, 390 replicates in corridor A after trawling, and 390 replicates in corridor B both before and after trawling.

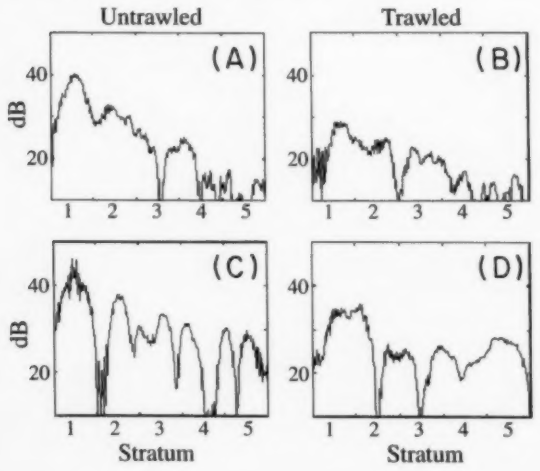


Fig. 6. Examples of single-element, Hilbert-transformed time histories for untrawled (A and C) and trawled (B and D) sites, highlighting comparisons in the five depth strata.

is the alteration of habitat structure. Until now, reliable data on the structure of the seabed at a scale of resolution relevant to the benthic biota have not been available. The present study provides strong evidence that trawling decreases the complexity of the habitat at this scale. The reduced fractals of post-trawling acoustic return signals are consistent to depths of 4.5 cm, an unexpected result in the hard-packed sand of the experimental area. The immediate structural changes in the benthic habitat that result from trawling raise the possibility of longer term impacts, especially if the trawling disturbance is chronic.

The significant decrease in the fractals of acoustic return signals from the trawled seabed compared with the pretrawled condition is an important result in that preliminary data on bulk sediment properties and biological community structure (T.W. Rowell, unpublished data) in the experiment, along with data from other studies on the impact of trawling, do not indicate dramatic immediate impacts related to trawling. However, the easily collected, high resolution, and data-rich DRUMS acoustic results reveal consistent structural differences on the scale of millimetres in the surface few centimetres of the sediment. Because the coherence of the return signal in the DRUMS system is high, and reverberation interference is virtually nonexistent, it has been possible to utilize the acoustic data, cast as fractals of the Hilbert-transformed time series,

Table 2. Treatment means and standard deviations of fractals by corridor and by trawl treatment within each corridor.

		Stratum 1		Stratum 2		Stratum 3		Stratum 4		Stratum 5	
Corridor	Trawl	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A		1.455	0.001 910	1.437	0.001 967	1.448	0.002 408	1.457	0.002 635	1.445	0.002 692
B		1.462	0.001 859	1.437	0.001 914	1.440	0.002 343	1.453	0.002 564	1.447	0.002 621
A	1	1.464	0.002 771	1.440	0.002 854	1.453	0.003 493	1.463	0.003 823	1.457	0.003 907
A	2	1.447	0.002 629	1.433	0.002 707	1.444	0.003 314	1.451	0.003 627	4.132	0.003 706
B	1	1.459	0.002 629	1.443	0.002 707	1.447	0.003 314	1.468	0.003 627	1.452	0.003 706
B	2	1.464	0.002 629	1.432	0.002 707	1.433	0.003 314	1.437	0.003 627	1.441	0.003 706

Note: For more details, see footnote to Table 1.

Table 3. Multivariate analysis of variance for effect of corridor (A or B) and trawling on the fractal value of high-resolution acoustic data.

	Test statistic	*F*	df	*P*
Corridor				
Wilk's λ	0.988 64	3.477	5, 1513	0.004
Lawley–Hotelling *U*	0.011 49	3.477	5, 1513	0.004
Pillai's *V*	0.011 36	3.477	5, 1513	0.004
Trawl (within corridor)				
Wilk's λ	0.944 10	8.829	10, 3026	0.000
Lawley–Hotelling *U*	0.058 45	8.838	10, 3024	0.000
Pillai's *V*	0.056 61	8.821	10, 3028	0.000

Note: For more details, see footnote to Table 1.

as an indicator of the fractal geometry of the sediment. The reduction of the fractals of the post-trawl time series data is statistically highly significant and leaves little doubt that the differences do not result from the large sample sizes alone. Preliminary video image analysis of a large number of frames taken by BRUTIV (bottom referencing underwater towed instrument vehicle) over trawled and untrawled corridors in this experiment (D.L. McKeown, Bedford Institute of Oceanography, unpublished data) corroborate the high resolution grab video, showing that the surface structure of the seabed was significantly altered after trawling. An overall smoothing effect was quantified by the reduction of video contrast of the trawled seabed in a gray-level analysis. The DRUMS results verify the video image analysis and show that the structural changes extend to at least 4–5 cm into the sediment.

The original intent of this work was to relate differences in fractals of trawled and untrawled sediments to differences in other biological and bulk sediment properties. Benthic faunal community analysis has not yet been completed for the trawling impact study, but preliminary results indicate that megafauna are reduced in number with successive trawl passes (unpublished data). J. Prena and T.W. Rowell (unpublished data) have not found strong evidence of immediate trawling impact on macrofaunal communities in their preliminary analyses. The acoustic measurements made by DRUMS, however, indicate a significant effect of trawling that we cannot say at this time will also be reflected in the final data on the infauna or sediment properties. Therefore, we present the acoustic data as indications in their own right of effects of trawling on the structure of a well-packed sandy seabed.

Physical disturbances of ecosystems result in loss of biological organization. While disturbed systems are physically degraded to a simpler abiotic structure with a greater random component, more complex, biogenically organized systems are characterized by high levels of structural and dynamic unpredictability, or chaos. Our experimental results demonstrate a reduction in this structural chaos, measured by a consistent and significant decrease in the fractal of high resolution acoustic return signals, in a sandy seabed physically disturbed by otter trawling. Further similar studies on cohesive and coarse sediments are required to produce a general model of the structural effects of physical disturbance on the seabed. In our analysis of chaos, we introduce a quantitative measure of the structural effects of physical disturbance on the benthic habitat.

Ecosystem function depends upon the exchange of materials and thus a maximum surface to volume ratio in the exchange structures, which in the benthos are the burrow walls, tubes, and galleries of the infauna (Rhoads 1974; Aller 1988; Pelegri and Blackburn 1994). A decrease in the fractal of such a system will result in a decrease in its exchange capacity. Animal structures in the sediment, like plant structures in terrestrial ecosystems, are designed to maximize the chaos of the system and thus maximize the efficiency of material and energy transfer. Therefore, the reduction of the fractal by a physical disturbance in an exchange system like the sandy marine seabed attacks the system in its primary functional role. The disturbance caused by experimental otter trawling, if chronic, may have profound impacts on the functioning of the whole ecosystem by reduction of exchange capacity. Reduction of fine-scale complexity by trawling indicates a much more basic disturbance of the benthic system than would be indicated if immediate mortality of some species were the most notable effect.

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References

Aller, R.C. 1988. Benthic fauna and biogeochemical processes in marine sediments: the role of burrow structures. *In* Nitrogen cycling in coastal marine environments. *Edited by* T.H. Blackburn and J. Sorensen. John Wiley and Sons Ltd., Chichester, U.K. pp. 301–338.

Barker, H.R., and Barker, B.M. 1984. Multivariate analysis of variance (MANOVA). University of Alabama, Tuscaloasa, Ala.

Berktay, H.O., and Leahy, D.J. 1974. Farfield performance of parametric transmitters. J. Acoust. Soc. Am. **55**: 539–546.

Beye, R.T. 1965. Nonlinear acoustics. *In* Physical acoustics, principles and methods. Vol. II. Part B. *Edited by* W.P. Mason. Academic Press, New York. pp. 231–264.

Bradbury, R.H., and Reichelt, R.E. 1983. Fractal dimension of a coral reef at ecological scales. Mar. Ecol. Prog. Ser. **10**: 169–171.

Gee, J.M., and Warwick, R.M. 1994. Metazoan community structure in relation to the fractal dimensions of marine macroalgae. Mar. Ecol. Prog. Ser. **103**: 141–150.

Guigné, J.Y., and Chin, V.H. 1989. Acoustic imaging of an inhomogeneous sediment matrix. Mar. Geophys. Res. **11**: 301–317.

Guigné, J.Y., Rukavina, N., Hunt, P.H., and Ford, J.S. 1991. An acoustic parametric array for measuring the thickness and stratigraphy of contaminated sediments. J. Great Lakes Res. **17**: 120–131.

Guigné, J.Y., Schwinghamer, P., Liu, P.Q., and Chin, V.H. 1993. High resolution and broadband processing of acoustic images of the marine benthos. *In* Proceedings of the Institute of Acoustics International Conference, Bath, U.K., 14–16 April, 1993. Vol. 15. Part 2. Acoustic Classification and Mapping of the Seabed. Bath University, Bath, U.K. pp. 237–252.

Jaggi, S., Quattrochi, D.A., and Lam, N.S.-N. 1993. Implementation and operation of three fractal measurement algorithms for analysis of remote-sensing data. Comput. Geosci. **19**: 745–767.

Kaye, B.H. 1989. A random walk through fractal dimensions. VCH Verlagsgesellschaft, Weinheim, Germany.

Kranenburg, C. 1994. The fractal structure of cohesive sediment aggregates. Estuarine Coastal Shelf Sci. **39**: 451–460.

Lewis, C.F.M., and Woodworth-Lynas, C.M.T. 1990. Ice source. *In* Geology of the continental margin of eastern Canada. Geology of Canada No. 2. *Edited by* M.I. Keen and G.L. Williams. Geological Survey of Canada, Ottawa, Ont. pp. 785–793.

Lu, S.Z., and Hellawell, A. 1994. An application of fractal geometry to complex microstructures. Acta Metall. Mater. **42**: 4035–4047.

Malinverno, A. 1990. A simple method to estimate the fractal dimension of a self-affine series. Geophys. Res. Lett. **17**: 1953–1956.

Mandelbrot, B.B. 1982. The fractal geometry of nature. W.H. Freeman, San Francisco, Calif.

Mellen, R.H., and Moffett, M.B. 1978. A numerical method for calculating the nearfield of a parametric acoustic source. J. Acoust. Soc. Am. **63**: 1622–1624.

Messieh, S.N., Rowell, T.W., Peer, D.L., and Cranford, P.J. 1991. The effects of trawling, dredging, and ocean dumping on the eastern Canadian continental shelf seabed. Cont. Shelf Res. **11**: 1237–1263.

Meyer, K., Lorenz, P., Bohlkuhn, B., and Klobes, P. 1994. Porous solids and their characterization. Cryst. Res. Technol. **29**: 903–930.

Minitab Inc. 1993. Minitab reference manual, release 9 for Windows. Sewers Printing Co., Lebanon, Pa.

Moffett, M.B., and Mellen, R.H. 1977. Model for parametric acoustic sources. J. Acoust. Soc. Am. **61**: 325–337.

Pelegrì, S.P., and Blackburn, T.H. 1994. Bioturbation effects of the amphipod *Corophium volutator* on microbial nitrogen transformations in marine sediments. Mar. Biol. (Berlin), **121**: 253–258.

Rhoads, D.C. 1974. Organism–sediment relations on the muddy sea floor. Oceanogr. Mar. Biol. Annu. Rev. **12**: 263–300.

Rowell, T.W., Chin-Yee, M., Steeves, G., Vass, W.P., Reimer, D., and Vine, R. 1994. Development and operational characteristics of a video augmented benthic grab and epibenthic sled. Report presented to ICES Working Group on the Ecosystem Effects of Fishing Activities, Copenhagen, 20–27 April, 1994, and to the Benthos Ecology Working Group, Yerseke, 10–13 May, 1994. Available from T.W. Rowell, Habitat Ecology Section, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, NS B2Y 4A2, Canada.

Rowell, T.W., Schwinghamer, P., Gilkinson, K., Gordon, D.C., Jr., Hartgers, E., Hawryluk, M., McKeown, D.L., Prena, J., Vass, W., and Woo, P. 1996. Investigating the impact of otter trawling on benthic communities of the Grand Bank. Can. Tech. Rep. Fish. Aquat. Sci. In press.

Urick, R.J. 1975. Principles of underwater sound. McGraw-Hill, New York.

Westervelt, P.J. 1963. Parametric acoustic array. J. Acoust. Soc. Am. **35**: 535–537.

Willette, J.G., and Moffett, M.B. 1977. Harmonics of the difference frequency in saturated-limited parametric sources. J. Acoust. Soc. Am. **62**: 1377–1381.

Wilson, D.K. 1993.Relaxation-matched modeling of propagation through porous media, including fractal pore structure. J. Acoust. Soc. Am. **94**: 1136–1145.

Spatial and taxonomic variation in incidence of mouthpart deformities in midge larvae (Diptera: Chironomidae: Chironomini)

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Abstract: Chironomids are potentially important indicators of the effects of sediment-bound contaminants. However, the group is taxonomically diverse, and sensitivity to pollutants varies among genera. We evaluated mouthpart (mentum) deformities of larval chironomids collected from six locations on the St. Clair and Detroit rivers, whose sediments varied in the amounts and types of organochlorine contaminants, to designate broadly distributed genera most suitable for indicating possible sediment teratogenicity. Two thousand and six larvae belonging to 18 genera of Chironomini were collected. Five genera were abundant and widely distributed among sites (*Chironomus*, *Cryptochironomus*, *Polypedilum*, *Stictochironomus*, *Phaenopsectra*). The incidence of deformities varied significantly among genera. *Cryptochironomus*, *Polypedilum*, and *Stictochironomus* showed uniformly low incidences of deformities at all sites (<3%). The incidences of deformities in two genera, *Chironomus* and *Phaenopsectra*, varied significantly from <2–3% at uncontaminated sites to 6–20% at contaminated locations. Within genera, deformities were more common in chironomids collected from locations with intermediate levels of contamination than from the most heavily contaminated site. A minimum sample size of ≥125 individuals is required to demonstrate that a doubling in the incidence of deformities of a genus over background levels would be significant 80% of the time.

Résumé : Les chironomidés peuvent être d'importants indicateurs des effets des contaminants liés aux sédiments. Toutefois, ce groupe est hétérogène sur le plan taxonomique, et sa sensibilité aux polluants varie selon les genres. Nous avons évalué les difformités du rostre (mentum) de larves de chironomidés provenant de six sites des rivières Sainte-Claire et Detroit, dont les sédiments contiennent des quantités et des types variables de contaminants organochlorés, afin de déterminer quels genres à large distribution sont les plus susceptibles de fournir des indications sur la tératogénicité des sédiments. On a recueilli 2006 larves appartenant à 18 genres de chironomidés. Cinq de ceux-ci étaient abondants et largement distribués parmi les sites (*Chironomus, Cryptochironomus, Polypedilum, Stictochironomus, Phaenopsectra*). L'incidence des difformités variait de façon significative d'un genre à l'autre. Les genres *Cryptochironomus, Polypedilum* et *Stictochironomus* présentaient des incidences uniformément faibles de difformités à tous les sites (moins de 3%). L'incidence des difformités chez deux genres, *Chironomus* et *Phaenopsectra*, présentait des variations significatives de moins de 2 à 3% aux sites non contaminés, et de 6 à 20% aux sites contaminés. À l'intérieur des genres, les difformités étaient plus communes chez les chironomidés recueillis en des lieux à niveaux intermédiaires de contamination qu'aux lieux les plus fortement contaminés. Un échantillon minimum de 125 individus ou plus est requis pour démontrer qu'un doublement de l'incidence des difformités d'un genre par rapport aux valeurs de fond serait significatif 80 fois sur cent.
[Traduit par la Rédaction]

Introduction

Chironomid larvae have excellent potential as aquatic biomonitor organisms as they live in intimate contact with sediments, feeding on detritus and algae associated with the sediments (Larsson 1984; Reynoldson 1987). The sediments are often reservoirs for pollutants (Pesch et al. 1981; Haffner et al. 1994). Chironomid larvae constitute a major component of the benthic invertebrate biomass associated with freshwater sediments and are often a significant portion of the diet of predatory invertebrates and fishes (Hart and Fuller 1974). Contaminants accumulated in invertebrate tissues are thus transmitted to foraging fish and waterfowl and ultimately to humans. Therefore, chironomid larvae potentially play a primary role in the bioaccumulation and transport of contaminants. Because chironomids are relatively sessile, they reflect the conditions in the sediments where they were collected (Pinder 1995).

Benthic invertebrates, including chironomid larvae, accumulate polynuclear aromatic hydrocarbons (PAHs) and numerous other persistent organochlorine compounds in their tissues (Knight 1984; Larsson 1984; Rosiu et al. 1989). Dickman et al. (1992) found that deformed chironomids had higher PAH concentrations in their tissues than did normal chironomids from the same site. They suggested that PAHs acted

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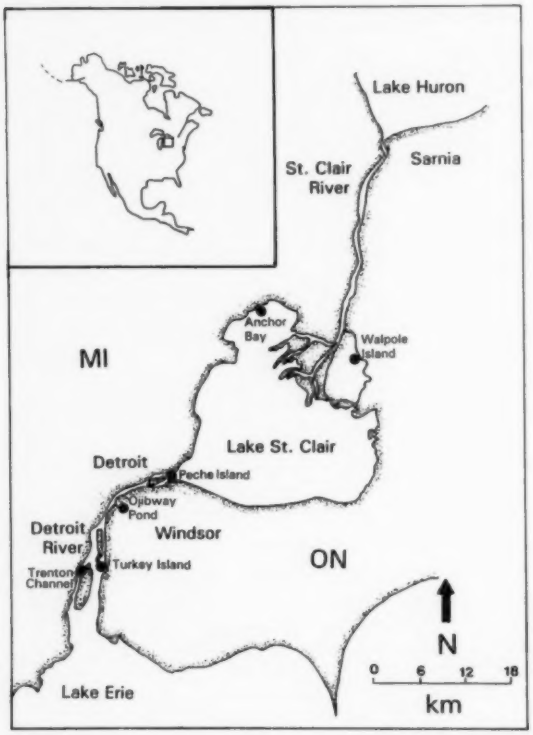
Fig. 1. Location of the study sites. The square in the inset figure of North America shows the general location of the study area.



as teratogens. Teratogenic abnormalities appear to result from the alteration of developing cells, which may lead to improper functioning of these cells and interference with differentiation such that proper cell types either do not form or do not develop in the proper number or location (Kamrin 1988). If this is the case, then elevated incidences of deformities in organisms at particular sites could indicate a biologically significant contaminant burden.

Morphological abnormalities in chironomids collected in the field have received increasing attention as potential indicators of environmental degradation. The most conspicuous features of deformed larvae are thickened exoskeletons and head capsules (Hamilton and Saether 1971), and various antennal (Warwick 1985, 1988, 1989, 1990*a*, 1990*b*, 1991; Dermott 1991) and mouthpart deformities (Dickman et al. 1990, 1992; van Urk et al. 1992; Diggins and Stewart 1993; Lenat 1993). Warwick (1985, 1989, 1990*b*, 1991) provided extensive documentation on types and severity of deformities that occur among different genera. Several researchers have addressed chironomid deformities in relation to environmental contaminant gradients (e.g., Hare and Carter 1976; Warwick 1988; van Urk et al. 1992; Diggins and Stewart 1993). Lenat (1993) compared composite incidences of deformities of one genus (*Chironomus*) from multiple stream locations varying in water quality.

Studies of chironomid deformities have commonly been hindered by small sample sizes and (or) lack of adequate spatial scale to permit a comprehensive evaluation of incidences across taxa and habitats. In some cases, the numbers of specimens available from a particular location and time are those recovered from a fixed number of area-delimited, quantitative bottom samples, i.e., sample size was determined by the number of collections, not necessarily the number of organisms required for a survey of different genera (van Urk et al. 1992; Diggins and Stewart 1993). Indeed, what constitutes an adequate sample size has not been well defined. Where special efforts have been made to collect large numbers of individuals (e.g., Dermott 1991; Bird 1994), samples were collected from relatively restricted geographic regions and (or) limited classes of environmental contamination.

Because there is great ecological and taxonomic diversity within the family Chironomidae, it is important to systematically assess variability in the susceptibility of different taxa to deformities. One objective of our study was to evaluate the variability in the incidence of deformities among taxa to determine which genera are most suitable for indicating sediment degradation. An appropriate genus should be easy to collect in large numbers across a broad geographic and environmental range. At the same time, it should be sensitive to induction of deformities, but only when exposed to environmental stress. A second objective of our study was to document spatial variation in the incidence of chironomid deformities in the St. Clair and Detroit rivers, and to correlate elevated incidence of deformities with specific types or levels of potentially teratogenic substances. We also wished to ascertain suitable minimum sample sizes necessary to demonstrate statistically significant deviations from background incidences: such estimates are contingent upon the magnitude of the effect being measured.

Materials and methods

Study areas

Larval chironomids were collected from depositional (fine) sediments at six study sites in the vicinity of Anchor Bay, Walpole Island, Peche Island, Ojibway Pond (Windsor), Turkey Island, and Trenton Channel in the Lake Huron – Lake Erie corridor (Fig. 1). These sites were chosen to provide a range of contaminant types and concentrations as part of an interdisciplinary study of sediment contamination effects (Ali et al. 1993; Haffner et al. 1994). Major contaminant inputs to the St. Clair – Detroit river systems are petrochemical by-products that enter the upper St. Clair River near Sarnia, Ontario, and diverse industrial chemicals and pesticides discharged into the Detroit River with waste water at Detroit, Michigan.

Anchor Bay was selected as an internal reference site. It has soft, muddy sediments similar in particle size distribution to the other sites (Griffiths 1987). However, the sediments are relatively uncontaminated (Pugsley et al. 1985) because most water from the St. Clair River flushes directly from the main river channels at Walpole Island and through the centre of Lake St. Clair to Peche Island (Leach 1980). Concentrations of polychlorinated biphenyls (PCBs) and octachlorostyrene (OCS) in Anchor Bay are among the lowest in the Huron–Erie corridor (Table 1). Pugsley et al. (1985) reported total PCB levels below the detection limit of 2.3 μg/kg and OCS levels less than 1 μg/kg.

An additional, external reference site (a 0.5-ha partly wooded, roadside pond at Ojibway Park near Windsor, Ont.) was sampled to provide baseline levels of deformities and document natural variability in mouthpart structure. Trace or nondetectable concentrations of petrochemicals and PCBs occur here (Table 1).

Table 1. Contaminant concentrations (mg/kg) in sediments collected from the six study sites.

Compound	Anchor Bay[a]	Walpole Island[b]	Peche Island[b]	Ojibway Pond	Turkey Island[c]	Trenton Channel[c]
Organochlorines						
QCB	nd	0.03	0.12	0.0001	0.02	0.30
HCB	nd	0.24	0.41	0.0002	0.05	1.72
OCS	nd	0.13	0.21	nd	0.01	0.37
p,p'-DDE	nd	0.02	1.00	0.004	0.02	1.02
Aroclor mix[d]	nd	0.23	0.94	0.014	0.27	12.53
Polynuclear aromatic hydrocarbons						
Naphthalene	na	nd	na	na	0.70	nd
Acenaphthylene	na	nd	na	na	nd	1.70
Acenaphthene	na	nd	na	na	nd	nd
Fluorine	na	0.36	na	na	nd	3.65
Phenanthrene	na	2.58	na	na	2.40	35.25
Anthracene	na	nd	na	na	nd	11.00
Fluoranthene	na	2.75	na	na	4.15	84.35
Pyrene	na	3.50	na	na	4.25	77.85
Benzo-[*a*]-anthracene	na	3.25	na	na	3.70	52.85
Chrysene–triphenylene	na	2.65	na	na	3.80	56.00
Benzo-[*a*]-fluoranthene	na	3.30	na	na	6.45	67.80
Benzo-[*k*]-fluoranthene	na	1.00	na	na	0.35	27.60
Benzo-[*a*]-pyrene	na	1.95	na	na	3.90	29.40
Indeno-[1,2,3,-*c,d*]-pyrene	na	nd	na	na	nd	25.55
Dibenzo-[*a,h*]-anthracene	na	nd	na	na	nd	13.40
Dibenzo-[*g,h,i*]-perylene	na	nd	na	na	nd	34.80

Note: Except for Anchor Bay, sediment samples on which contaminant analyses are based were collected during chironomid surveys. nd, not detected; na, not available.

[a]Data from Pugsley et al. (1985).

[b]Detailed information in Haffner et al. (1994).

[c]Detailed information in Ali et al. (1993).

[d]PCBs summarized here as 1:1 mixture of Araclors 1254 and 1260.

The Walpole Island site is located in the Chenal Ecarte of the St. Clair River approximately 50 km downstream of the Sarnia industrial waterfront (Fig. 1). Sediments and emergent aquatic insects are moderately contaminated, primarily with petrochemical industrial by-products including hexachlorobenzene (HCB), OCS, and pentachlorobenzene (QCB) (Kovats and Ciborowski 1989, Table 1).

The Peche Island site is at the head of the Detroit River (Fig. 1). Sediment-bound petrochemicals such as HCB are transported here from the St. Clair River (Environment Canada and the U.S. Environmental Protection Agency 1988). Sediments from this area have relatively higher concentrations of several contaminants, including PCBs and petrochemicals, than the Walpole Island site (Table 1).

The Trenton Channel and Turkey Island sites in the Detroit River were chosen to illustrate local spatial differences in the Detroit River sediments. Thornley and Hamdy (1984), Kauss and Hamdy (1985), and the International Joint Commission (1987) reported considerably higher sediment PCB concentrations at the Trenton Channel site than at Turkey Island. Recent data support this (Table 1).

Anchor Bay and Ojibway Park, the two reference sites, were expected to yield larvae with the lowest incidences of deformities. We anticipated that chironomids from Walpole and Turkey islands would display elevated but similar incidences of deformities. Incidences of deformities in organisms from the Peche Island site were expected to be greater than this. Finally, on the basis of sediment contaminant levels, the Trenton Channel organisms were anticipated to display the greatest incidence of deformities.

Study animals and collection procedures

Between May 1991 and May 1993, the six study sites were sampled using either a 15 × 15 cm petite Ponar grab sampler or a 30-cm wide D-frame kick net, depending upon the water depth at the site being sampled. Samples were taken 1–20 m from shore at depths of 1–15 m. Each site was visited three times during spring–summer (May–August). Our goal was to acquire at least 300 larvae per site: on each visit, up to 250 sediment samples were collected at each site.

Upon collection, sediments were transferred to and rinsed through a sieve bucket (mesh size 0.600 mm), which rendered chironomid larvae readily visible. We restricted our collections to the visible, red (hemoglobin containing) chironomids (primarily Chironomini) because these genera are characteristic inhabitants of hypoxic, depositional regions, where sediment-bound contaminants are most likely to accumulate. Because Chironomini are largely detrital feeders (Coffman and Ferrington 1984), they are exposed to hydrophobic contaminants in the sediment through their diet in addition to direct exposure through contact with overlying and pore water. The larger (visible) individuals were removed from the sieve bucket with forceps, blotted on paper towelling to remove excess water, and preserved in vials of chilled Carnoy's fluid (3:1 (v/v) absolute ethanol – glacial acetic acid).

Mounting and evaluation

In the laboratory, larvae were individually mounted on slides for taxonomic identification and morphological examination. Only third- and fourth-instar larvae were mounted because smaller individuals cannot reliably be identified to the generic level (Oliver and Roussell 1983).

The head capsule of each larva was removed and placed ventral side up on a microscope slide in a drop of CMC-9AF aqueous mounting medium (Master's Chemical Company, Des Plaines, Ill.).

Table 2. Number of chironomid larvae of each genus examined from the six study sites.

Genus	Anchor Bay	Ojibway Pond	Walpole Island	Peche Island	Turkey Island	Trenton Channel
Chironomus	49	283	26	305	79	0
Phaenopsectra	118	0	11	24	133	45
Stictochironomus	174	0	126	3	17	0
Polypedilum	1	0	230	35	19	0
Cryptochironomus	29	0	43	63	68	2
Microtendipes	0	0	1	40	0	0
Endochironomus	0	20	0	0	0	0
Harnischia	0	0	14	0	0	0
Demicryptochironomus	8	0	3	0	0	0
Einfeldia	3	4	0	1	2	0
Dicrotendipes	0	0	0	1	9	0
Pseudochironomus	8	0	0	0	0	0
Glyptotendipes	0	4	0	0	1	0
Cladopelma	0	0	0	2	0	0
Nilodorum	0	0	2	0	0	0
Paratendipes	0	0	1	0	1	0
Nilothauma	1	0	0	0	0	0
Parachironomus	0	0	0	1	0	0
Total	391	317	457	475	329	47

Note: Genera are listed in declining order of overall abundance.

The corresponding body of each individual was stored in a labelled shell vial containing Carnoy's fluid for later analysis of polytene chromosome structure (Hudson 1994). A cover slip was placed on the slide and gentle pressure was applied to separate the mouthparts and properly orient the head capsule. The cover slip was then ringed with clear nail polish and allowed to dry and clear for at least 7 d. Chironomids were identified to genus using the keys of Oliver and Roussell (1983), Wiederholm (1983), and Coffman and Ferrington (1984).

Each head capsule was examined for the presence of mentum deformities. A mentum was classified as either normal or deformed on the basis of the criteria of Dickman et al. (1992). Our designation of deformed corresponds with deformity classes II and III of Lenat (1993). A mentum that exhibited blunt or chipped teeth was judged to be worn (normal) but not deformed. Similarly, a mentum tooth with jagged edges was considered to be broken and also was classified as normal. Only misshapen teeth on the mentum that had smooth edges were considered to be deformed. Other characteristics of the mentum that resulted in a classification of deformed included fused teeth, crossed teeth, extra teeth, missing teeth, or teeth of aberrant shape or size. Asymmetry of the mentum was also an indication of deformity. After the examination for deformities in all chironomids, each deformed specimen was reexamined to confirm that it had been categorized correctly. Characteristic deformities of the mouthparts have been previously illustrated elsewhere (Dermott 1991; Diggins and Stewart 1993; Bird 1994).

Statistical methods

The incidence of deformed larvae from the different study sites was expressed as the proportion ±1 standard error (SE) of larvae at each site that displayed deformities. SEs were calculated according to the binomial theorem, i.e., $SE = (pq/k)^{1/2}$, where p is the proportion of deformed specimens, q is the proportion of undeformed specimens, and k is the sample size (Sokal and Rohlf 1981). The G statistic goodness of fit test (Sokal and Rohlf 1981) was used to test for heterogeneity in the incidence of deformities among sites (H_0: incidence of deformities is equal at all sites) and among genera (H_0: incidence of deformities is equal among all genera).

Results

A total of 2006 red chironomids, belonging to 18 genera (all Chironomini) were examined from the six designated study sites (Table 2). Representatives of only six of these genera (*Chironomus*, *Cryptochironomus*, *Microtendipes*, *Polypedilum*, *Stictochironomus*, and *Phaenopsectra*) occurred in large enough numbers at one or more sites to be considered common (40 or more individuals).

Microtendipes was common at only a single site. The remaining common genera each occurred at four or more locations. Only two genera (*Cryptochironomus* and *Phaenopsectra*) were found at the heavily contaminated Trenton Channel site. Few invertebrates of any description except for oligochaetes were collected from this location.

The most common type of deformity observed was asymmetry in the number of lateral teeth of the mentum (usually loss of one or more teeth on either side of the median tooth). Occurrence of extra lateral teeth was the second most common deformity. Relatively few deformities were associated with the median tooth, and gaps in the mentum were very rare. There was no indication that specific types of deformities were associated with specific locations or genera: missing teeth, extra teeth, and asymmetry in mentum shape were present in chironomid larvae collected from both uncontaminated and contaminated sites.

There was significant heterogeneity in the overall incidence of mentum deformities among the five widespread, common genera (G statistic goodness of fit test, $G = 43.68$, $p < 0.001$). *Cryptochironomus*, *Polypedilum*, and *Stictochironomus* exhibited a low overall incidence of deformities, ranging from 0 to 2.5% (Table 3). The other two abundant genera, *Chironomus* and *Phaenopsectra*, were more prone to deformities, with overall incidences of 9.3 and 5.7%, respectively.

There was among-site variation in the incidence of mentum deformities for the two genera displaying the greatest overall

Table 3. Incidences of mentum deformities (percentage ± 1 SE) of the most abundant genera at individual study sites and all sites combined.

Genus	Anchor Bay	Ojibway Pond	Walpole Island	Peche Island	Turkey Island	Trenton Channel	Overall incidence
Most susceptible to deformities							
Phaenopsectra	1.7±1.2	na	18.2±12.8	0.0±0.0	9.0±2.5	6.7 ±3.7	5.7±1.3
	(118)	(0)	(11)	(24)	(133)	(45)	(331)
Chironomus	2.0±2.0	3.2±1.0	19.2±7.7	16.7±2.1	3.8±2.2	na	9.3±1.1
	(49)	(283)	(26)	(305)	(79)	(0)	(742)
Least susceptible to deformities							
Cryptochironomus	0.0±0.0	na	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	(29)	(0)	(43)	(63)	(68)	(2)	(205)
Polypedilum	0.0±0.0	na	1.3±0.7	0.0±0.0	5.3±5.1	na	1.4±0.7
	(1)	(0)	(230)	(35)	(19)	(0)	(285)
Stictochironomus	1.1±0.8	na	4.8±1.9	0.0±0.0	0.0±0.0	na	2.5±0.9
	(174)	(0)	(126)	(3)	(17)	(0)	(320)

Note: Sample size is in parentheses. Overall incidence of deformities was genus specific (G statistic goodness of fit test, $G = 43.678$, $p < 0.001$).

frequency of deformities (G statistic goodness of fit test, $G = 43.27$, $p < 0.001$; and $G = 10.77$, $p < 0.05$ for *Chironomus* and *Phaenopsectra*, respectively). For *Chironomus*, deformities were most common in populations from the Walpole and Peche island sites and least common in the two reference site populations (Table 3). No specimens were collected from Trenton Channel. The Walpole Island *Phaenopsectra* population also displayed maximum incidences of deformity (Table 3). No deformities were detected in individuals from Peche Island, but relatively few individuals of the sensitive genera were collected from both Walpole Island and Peche Island (Table 2). *Phaenopsectra* was absent from the Ojibway Pond reference site.

Discussion

To be useful as a field biomonitor, a taxon should be both widespread and sensitive to changes in environmental quality. The ubiquity of Chironomidae makes them especially promising for such studies. Our collection of visually distinctive, large, red chironomids yielded 18 genera, 5 of which were abundant and widespread enough to warrant further consideration as generalized bioindicators of deformities. Our samples were clearly size biased by our procedure of handpicking living larvae retained in coarse-mesh sieves. However, this ensured that older larvae, which would have had extended exposure to sediments, were preferentially selected. Previously, only the tanypodine chironomid *Procladius* had been collected in sufficient numbers and ecological breadth to be rigorously evaluated for variability in incidence of deformities (Dermott 1991).

Chironomini, especially *Chironomus*, occupy depositional habitats ranging from oligotrophic to eutrophic (Saether 1979). *Chironomus* is the only widespread genus that occurs in streams of different sizes and water quality (Lenat 1993), and across rivers that vary widely in the types and concentrations of contaminants (Dermott 1991; Dickman et al. 1992; Diggins and Stewart 1993; Bird 1994).

The genera that we found to be widespread have been recognized previously as contaminant tolerant. Dickman et al. (1992) reported that *Chironomus*, *Endochironomus*, and *Polypedilum* maintain populations at contaminated sites where other genera cannot survive. Dermott (1991) collected *Phaenopsectra* (*Tribelos*) *jucundus* and *Cryptochironomus* from sites degraded by contaminants. *Cryptochironomus* and *Phaenopsectra* were the only genera that we collected at all nonreference sites, including the most heavily contaminated one (Trenton Channel). The low susceptibility of these genera, and of *Stictochironomus*, to mentum deformities in our study attests to their ability to withstand contaminated conditions. However, this resistance reduces their suitability as biomonitors.

Our study considered only mentum deformities. Much of the detailed work on chironomid deformities has involved examining the antennae. Although Warwick (1985) elegantly demonstrated that chironomid antennal deformities are a sensitive index of pollution, Dermott (1991) observed that antennae are more easily damaged during sampling or distorted during mounting than are hardened mouthparts. The optical resolution necessary to properly discern antennal fine structure requires more careful clearing and orientation during mounting, and examination at higher magnification than is needed to evaluate the mentum. Dermott (1991) reported that a week was required to collect, sort, and mount 300 larvae, and about 3 min per specimen to scan for deformities of various body structures (anal papillae, antennae, various head structures). In contrast, excluding collection time, we could mount 300 larvae in 4 d and required approximately 1 min per specimen to score the mentum. Thus, examining only the mentum increases efficiency and allows greater sample sizes to be examined (where available). However, Janssens de Bisthoven et al. (1995) recently found independent correlations between contaminants associated with antennal deformities and those related to mentum deformities, suggesting that each measure could provide unique information.

Chironomus and *Phaenopsectra* exhibited the greatest incidences of mentum deformities. Dermott (1991) also reported elevated incidences of deformities in *Chironomus* and *Phaenopsectra* (30 and 9%, respectively) from the St. Clair River. Dermott (1991) and Warwick and Tisdale (1988) suggested that the susceptibility of *Chironomus* to deformities (10–15× greater than that of the pollutant-tolerant, predatory genus *Procladius*) might be attributable to *Chironomus* feeding directly on contaminant-laden detritus. Diggins and

Stewart (1993) reported that 29% of *Chironomus* larvae collected from the heavily metal-contaminated Buffalo River had deformities, whereas only 15% of *Procladius* larvae displayed aberrant mouthparts. They, too, found that deformities in *Cryptochironomus* (also a predator) and *Polypedilum* were rare. Wiederholm (1984) noted that in areas having a high incidence of deformity in some genera, cohabiting genera did not display deformities.

Several recent studies have reported approximately linear elevations in deformity incidences along gradients of contamination (e.g., Janssens de Bisthoven et al. 1995). Van Urk et al. (1992), Dickman et al. (1992), Bird (1994), and Madden et al. (1995) found increasing frequencies of deformities as levels of metals, coal tar, urban or agricultural runoff, and pesticides rose, respectively.

Although deformities were more common in larvae collected from our contaminated sites than from reference locations, the correspondence between frequency of deformities and gross level of contamination was disappointing. We expected that organisms collected from the highly contaminated Trenton Channel would display the greatest incidences of deformities. However, Walpole Island organisms were most prone to deformities. Perhaps the animals most severely affected at the most contaminated sites die and are therefore not collected (Warwick 1990*a*).

Although incidences of deformities were significantly greater at contaminated than at the reference sites, we could not demonstrate clear differences among contaminated sites. The deformity frequency at Turkey Island was elevated for *Phaenopsectra* (equivalent to the frequency at Trenton Channel), but incidences in *Chironomus* larvae from this site were equivalent to background levels. In contrast, deformity levels in Peche Island *Chironomus* approached those at Walpole Island, whereas no deformities at all were found among the few Peche Island *Phaenopsectra* that we examined. These inconsistencies are partly explained by the relatively small sample sizes at some sites. The presence or absence of a single deformed larva in a small sample creates large changes in proportions. Additionally, sensitivity to particular chemicals may be taxon specific. Such apparent taxon specificity increases the potential value of chironomid deformities in discriminating among classes of contaminants but it underlines the need for species- and contaminant-specific controlled dose–response studies. Conclusive laboratory cause–effect data are largely still lacking. Hamilton and Saether (1971) found no induced deformities in *Chironomus tentans* larvae exposed to sublethal amounts of insecticides, herbicides, and PCBs in the laboratory. Madden et al. (1995) reported the frequencies of mentum deformities induced in bioassay tests by DDT and lead to be a nonlinear function of concentration. Hudson and Ciborowski (1996) demonstrated a log-linear relationship between incidence of deformities in *Chironomus* gp. *salinarius* larvae and bulk amounts of whole Trenton Channel sediment diluted with an uncontaminated formulated sediment, but individual compounds were not investigated.

Our results relating numbers of deformities to degree of contaminant among nonreference sites are inconclusive for *Phaenopsectra* and *Chironomus* partly because of small sample sizes for these genera at some sites. Clearly, the ability to detect potential differences in incidence of deformities among sites (statistical power) depends on obtaining an adequate sample size from each location. In our study, deformities were at least twice as common at contaminated impact locations (≥6%) as at the two reference stations (≤3%). Dermott (1991) found similar effects of contamination on ligula deformities in *Procladius*. Lenat (1993) also reported the overall incidence of *Chironomus* deformities among 21 clean-water stream sites as <3%. For a doubling in the incidence of deformities over 3% background levels to be judged significant ($p < 0.05$) with a power of 80%, at least 125 larvae from each population must be collected (sample size determined from binomial theorem, Sokal and Rohlf 1981). This can be difficult or, as at the Trenton Channel location, impossible when one is confronted with a sample site that is so heavily contaminated that impacts are already evident in reduced abundance and altered community structure. Use of a sensitive biomonitoring procedure, such as examining menta for deformities, should be unnecessary in such grossly affected regions. However, the greatest utility of such surveys is to provide evidence of subtle environmental degradation before gross effects at the community level become obvious. (Saether 1970).

In summary, we have identified both spatial and taxonomic variation in the incidence of chironomid mentum deformities. Five genera of Chironomini were found at sites that were widespread both geographically and across a diverse gradient of contamination. Only two of the genera display adequate variation in incidence of deformities. We recommend *Chironomus* and *Phaenopsectra*, together with the previously proposed genus *Procladius* (Dermott 1991), as being both broadly distributed and sensitive enough for use in deformity studies. However, minimum sample sizes of 125 individuals per genus are required to provide statistical power adequate to detect a significant elevation (doubling) of deformities over background. Given the spatial variation in community composition and finite sampling resources, sample sizes this large may not be achievable. Therefore, censuses of mentum deformities may have limited applicability in identifying sites that have subtle contamination differences. Controlled dose–response studies are necessary to quantify the differential sensitivity of taxa to specific classes of environmental contaminants.

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References

Ali, F., Lazar, R., Haffner, G.D., and Adeli, K. 1993. Development of a rapid and simple genotoxicity assay using a brown bullhead fish cell-line: application to toxicological surveys of sediments in the Huron–Erie corridor. J. Great Lakes Res. **19**: 342–351.

Bird, G.A. 1994. Use of chironomid deformities to assess environ-

mental degradation in the Yamaska River, Quebec. Environ. Monit. Assess. **30**: 163–175.

Coffman, W.P., and Ferrington, L.C. 1984. Chironomidae. *In* An introduction to the aquatic insects of North America. 2nd ed. *Edited by* R.W. Merritt and K.W. Cummins. Kendall Hunt Publishing Co., Dubuque, Iowa. pp. 551–652.

Dermott, R.M. 1991. Deformities in larval *Procladius* spp. and dominant Chironomini from the St. Clair River. Hydrobiologia, **219**: 171–185.

Dickman, M., Lan, Q., and Matthews, B. 1990. Teratogens in the Niagara River watershed as reflected by chironomid (Diptera: Chironomidae) labial plate deformities. Water Pollut. Res. J. Can. **24**: 47–79.

Dickman, M., Brindle, I., and Benson, M. 1992 . Evidence of teratogens in sediments of the Niagara River watershed as reflected by chironomid (Diptera: Chironomidae) deformities. J. Great Lakes Res. **18**: 467–480.

Diggins, T.P., and Stewart, K.M. 1993. Deformities of aquatic larval midges (Chironomidae: Diptera) in the sediments of the Buffalo River, New York. J. Great Lakes Res. **19**: 648–659.

Environment Canada and the U.S. Environmental Protection Agency. 1988. Upper Great Lakes connecting channels study. Final report. Vol. II. U.S. Environmnetal Protectection Agency, Chicago, Ill.

Griffiths, R.W. 1987. Environmental quality assessment of Lake St. Clair in 1983 as reflected by the distribution of benthic invertebrate communities. Prepared for the Ontario Ministry of the Environment. Aquatic Ecostudies, Ltd., Kitchener, Ont.

Haffner, G.D., Tomczak, M., and Lazar, R. 1994. Organic contaminant exposure in the Lake St. Clair food web. Hydrobiologia, **281**: 19–28.

Hamilton, A.C., and Saether, O.A. 1971. The occurrence of characteristic deformities in the chironomid larvae of several Canadian lakes. Can. Entomol. **103**: 363–368.

Hare, L., and Carter, J.C.H. 1976. The distribution of *Chironomus* (*s.s*)? *cucini* (*salinarius* group) larvae (Diptera: Chironomidae) in Parry Sound, Georgian Bay, with particular reference to structural deformities. Can. J. Zool. **54**: 2129–2134.

Hart, C.N., and Fuller, S.L.H. 1974. Pollution ecology of freshwater invertebrates. Academic Press, New York.

Hudson, L.A. 1994. Chironomid larvae (Diptera: Chironomidae) as indicators of sediment teratogenicity and genotoxicity. M.Sc. thesis, University of Windsor, Windsor, Ont.

Hudson, L.A., and Ciborowski, J.J.H. 1996. Teratogenic and genotoxic effects of contaminated sediment on midge (*Chironomus salinarius* gp.) larvae (Diptera: Chironomidae). Environ. Toxicol. Chem. In press.

International Joint Commission. 1987. Guidance on characterization of toxic substances problems in Areas of Concern in the Great Lakes basin. International Joint Commission, Windsor, Ont.

Janssens de Bisthoven, L., Huysmans, C., and Ollevier, F. 1995. The in situ relationships between sediment concentrations of micropollutants and morphological deformities in *Chironomus* gr. *thummi* larvae (Diptera, Chironomidae) from lowland rivers (Belgium): a spatial comparison. *In* Chironomids: from genes to ecosystems. *Edited by* P. Cranston. CSIRO Publications, East Melbourne, Australia. pp. 63–80.

Kamrin, M.A. 1988. Toxicology. Lewis Publishers, Inc., Chelsea, Mich.

Kauss, P.B., and Hamdy, Y. 1985. Biological monitoring and organochlorine contaminants in the St. Clair and Detroit Rivers using introduced clams *Elliptio complanatus*. J. Great Lakes Res. **11**: 247–263.

Knight, A.W. 1984. The evaluation of contaminated sediments employing selected benthic freshwater invertebrates. Final report. USEPA Cooperative Agreement No. CR-808424, University of California, Davis, Calif. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, Oreg.

Kovats, Z.E., and Ciborowski, J.J.H. 1989. Aquatic insect adults as indicators of organochlorine contamination. J. Great Lakes Res. **15**: 623–634.

Larsson, P. 1984. Transport of PCBs from aquatic to terrestrial environments by emerging chironomids. Environ. Pollut. **34**: 283–289.

Leach, J.H. 1980. Limnological sampling intensity in Lake St. Clair in relation to distribution of water masses. J. Great Lakes Res. **6**: 141–145.

Lenat, D.R. 1993. Using mentum deformities of *Chironomus* larvae to evaluate the effects of toxicity and organic loading in streams. J. North Am. Benthol. Soc. **12**: 265–269.

Madden, C.P., Austin, A.D., and Suter, P.J. 1995. Pollution monitoring using chironomid larvae: What is a deformity? *In* Chironomids: from genes to ecosystems. *Edited by* P. Cranston. CSIRO Publications, East Melbourne, Australia. pp. 89–100.

Oliver, D.R., and Roussell, M.E. 1983. The insects and arachnids of Canada. Part II. The genera of larval midges of Canada. Diptera: Chironomidae. Agric. Can. Publ. No. 1746.

Pesch, G.G., Pesch, C.E., and Malcolm, A.R. 1981. *Neanthes arenaceodentata*, a cytogenic model for marine genetic toxicology. Aquat. Toxicol. 1981: 301–311.

Pinder, L.C.V. 1995. The habitats of chironomid larvae. *In* The Chironomidae: biology and ecology of non-biting midges. *Edited by* P.D. Armitage, P.S. Cranston, and L.C.V. Pinder. Chapman and Hall, London, U.K. pp. 107–135.

Pugsley, C.W., Hebert, P.D.N., Wood, G.W., Brotea, G., and Obal, T.W. 1985. Distribution of contaminants in clams and sediments from the Huron–Erie corridor. I. PCBs and octachlorostyrene. J. Great Lakes Res. **11**: 275–289.

Reynoldson, T.B. 1987. Interactions between sediment contaminants and benthic organisms. Hydrobiologia, **149**: 53–66.

Rosiu, C.J., Giesy, J.P., and Kreis, R.G., Jr. 1989. Toxicity of sediments in the Trenton Channel, Detroit River, Michigan to *Chironomus tentans* (Insecta: Chironomidae). J. Great Lakes Res. **15**: 570–580.

Saether, O.A. 1970. A survey of the bottom fauna in lakes of the Okanagan Valley, British Columbia. Fish. Res. Board Can. Tech. Rep. No. 196. pp. 1–33.

Saether, O.A. 1979. Chironomid communities as water quality indicators. Holarct. Ecol. **2**: 65–74.

Sokal, R.R., and Rohlf, J. 1981. Biometry. 2nd ed. W.H. Freeman and Co., New York.

Thornley, S., and Hamdy, Y. 1984. An assessment of the bottom fauna and sediments of the Detroit River. Ontario Ministry of the Environment, Toronto., Ont.

van Urk, G., Kerkum, F.C.M., and Smit, H. 1992. Life cycle patterns, density, and frequency of deformities in *Chironomus* larvae (Diptera: Chironomidae) over a contaminated sediment gradient. Can. J. Fish. Aquat. Sci. **49**: 2291–2299.

Warwick, W.F. 1985. Morphological abnormalities in Chironomidae (Diptera) larvae as measures of toxic stress in freshwater ecosystems: indexing antennal deformities in *Chironomus* Meighen. Can. J. Fish. Aquat. Sci. **42**: 1881–1914.

Warwick, W.F. 1988. Morphological deformities in Chironomidae (Diptera) larvae as biological indicators of toxic stress. *In* Toxic contaminants and ecosystem health: a Great Lakes focus. *Edited by* M.S. Evans. Wiley and Sons, New York. pp. 281–320.

Warwick, W.F. 1989. Morphological deformities in larvae of *Procladius* Skuse (Diptera: Chironomidae) and their biomonitoring potential. Can. J. Fish. Aquat. Sci. **46**: 1255–1271.

Warwick, W.F. 1990*a*. The use of morphological deformities in chironomid larvae for biological effects monitoring. Sci. Ser. Inland Water/Lands Dir. Can. No. 173. pp. 1–34.

Warwick, W.F. 1990*b*. Morphological deformities in Chironomidae (Diptera) larvae from Lac St. Louis and Laprairie Basins of the St. Lawrence River. J. Great Lakes Res. **16**: 185–208.

Warwick, W.F. 1991. Indexing deformities in ligulae and antennae of *Procladius* larvae (Diptera:Chironomidae): application to contaminant-stressed environments. Can. J. Fish. Aquat. Sci. **48**: 1151–1166.

Warwick, W.F., and Tisdale, N.A. 1988. Morphological deformities in *Chironomus*, *Cryptochironomus*, and *Procladius* larvae (Diptera: Chironomidae) from two differentially stressed sites in Tobin Lake, Saskatchewan. Can. J. Fish. Aquat. Sci. **45**: 1123–1144.

Wiederholm, T. (*Editor*). 1983. Chironomidae of the holarctic region. Keys and diagnoses. Part I. Larvae. Entomol. Scand. Suppl. **19**: 1–457.

Wiederholm, T. 1984. Incidence of deformed chironomid larvae (Diptera: Chironomidae) in Swedish lakes. Hydrobiologia, **109**: 243–249.

Interactions between age-classes of Atlantic cod and their distribution among bottom substrates

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Abstract: When settling out of the water column, age 0+ Atlantic cod (*Gadus morhua*) enter a habitat already occupied by older conspecifics. To elucidate the nature of possible interactions between age-classes, we examined the influence of conspecifics on use of two combinations of bottom substrates by age 0+ and 1+ cod: sand and cobble, and sand and gravel. Substrate use by age 0+ and 1+ cod was examined in the absence and presence of a predator (age 3+ cod), when each age-class was either alone or together in the laboratory tank. Regardless of substrate combination, age 0+ and 1+ cod used sand when there was no predator. However, with a predator, both 0+ and 1+ cod either avoided the predator (sand and gravel) or hid in the cobble. Substrate use by the age 1+ cod was the same when together with age 0+ cod as when alone; in contrast, age 0+ fish avoided age 1+ fish, resulting in a significant increase in use of gravel or cobble. When a predator was present, avoidance of age 1+ cod by age 0+ cod disappeared; the presence of age 1+ cod significantly reduced predation on age 0+ fish. Our results suggest that some level of habitat segregation should be expected between age 0+ and 1+ cod.

Résumé : Les morues pélagiques (*Gadus morhua*) (classe d'âge 0+) s'installent dans les régions côtières où des congénères de plus grande taille sont déjà installé(e)s. Pour élucider les méchanismes possibles d'interactions entre ces deux groupes d'âge, nous avons examiné, en laboratoire, les effets d'un prédateur congénère (classe d'âge 3+) sur la sélection du type de substrat des jeunes morues. Nous avons offert aux jeunes morues deux combinaisons de substrats : du sable et des roches, et du sable et des gravillons. Nous avons testé le choix de substrat des jeunes morues en l'absence et en la présence l'une de l'autre. Peu importe la combinaison des types de substrats, individuellement, les deux classes de morues (classes d'âge 0+ et 1+) préféraient le sable en l'absence du prédateur. Par contre, en présence d'un prédateur, individuellement, les deux jeunes classes d'âge évitaient le prédateur lorsque la combinaison de substrats était sable et gravier, ou se cachaient sous les roches. Lorsque les deux classes d'âge étaient en présence l'une de l'autre, la sélection du type de substrat est resteé inchanger pour les morues d'un an. Par contre, les morues de l'année évitaient les morues d'un an, résultant en l'augmentation de l'usage du gravier ou des roches. En présence du prédateur, la compétition entre les deux classes d'âge disparaissait. En fait, la présence des morues d'un an a significativement réduit le taux de prédation des morues de l'anneé. À partir de nos résultats, il semble que les jeunes morues (classes d'âge 0+ et 1+) partageraient leurs habitats.

Introduction

The life history of numerous aquatic organisms involves a pelagic larval stage, followed by a shift to a demersal or benthic existence in the juvenile and adult stage. This life-history pattern requires a dramatic ontogenic habitat shift between the larval and juvenile stages, and if survival of the juveniles is habitat dependent, factors that may limit access to suitable habitat might have a negative impact on recruitment to the adult population (e.g., Carr 1994). For example, following their pelagic egg, larval, and juvenile stages, age 0+ Atlantic cod settle out of the water column and assume a demersal existence. Although juvenile cod seem to settle on a number of different bottom types (Tupper and Boutilier 1995), subsequent distribution and survival appear to be habitat dependent (Lough et al. 1987). If suitable habitat is in some way limited for age 0+ cod, the period between settlement and becoming 1-year-old juveniles could represent a bottleneck in cod recruitment.

A number of factors may limit the availability of suitable habitat to an organism. Most simply, habitat may be limited by its physical abundance (Anderson 1994; Carr 1994). It is also possible that preferred habitat may already be occupied by competitors. In Newfoundland, age 0+ cod are known to settle in inshore areas, an environment already occupied by 1-year-old conspecifics. Age 0+ and 1-year-old cod have been captured in the same sample in inshore environments in Newfoundland (Keats et al. 1987; Methven and Bajdik 1994; S. Fraser, V. Gotceitas, and J.A. Brown, personal observations) and elsewhere (Riley and Parnell 1984; Nordeide and Fosså 1992). These results suggest that these age-classes may coexist in similar inshore habitats. However, most sampling procedures used to collect juvenile cod typically cover an extensive area, and may therefore sample a variety of different habitat types at any one time. If so, the occurrence of various age-classes of cod in the same sample may not necessarily indicate similar habitat use by these fish (Gjosæter 1987).

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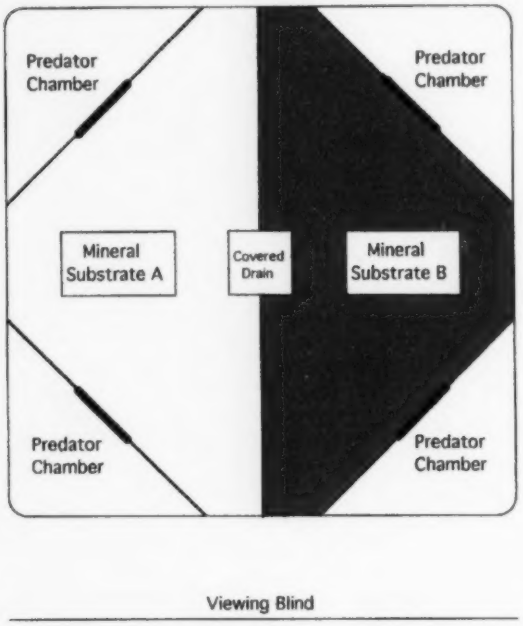
Fig. 1. Top view of experimental tank (See methods for details).



While various abiotic factors influence age-specific distribution of Atlantic cod (e.g., Wigley and Serchuck 1992), to our knowledge, few studies have examined the effects of intraspecific interactions. Studies looking at diet and choice of foraging sites suggest that competition among and within age-classes of juvenile cod may exist (Nordeide and Fosså 1992; van Duren and Glass 1992). In addition, cod are cannibalistic (Gjosæter 1987; Bogstad et al. 1994; S. Fraser, V. Gotceitas, and J.A. Brown, personal observations), and the risk of predation from older conspecifics has been shown to influence substrate use by juveniles (Gotceitas and Brown 1993) and may even be responsible for large-scale (inshore versus offshore) segregation between juvenile and adult cod (Bogstad et al. 1994). However, despite the fact that they appear to coexist in the inshore environment, it is not known whether intraspecific interactions between age-classes of juvenile cod affect their habitat use. Recently, Methven and Bajdik (1994) referred to a study on the abundance of young cod along the coast of Finland and Denmark, where it has been suggested that the limited numbers of age 0+ cod found in the nearshore environment may be due to competition and predation from larger cod (age 1+ and 2+ conspecifics), as well as other species of finfish. Therefore, we conducted a laboratory experiment to examine whether intraspecific interactions between age 0+ and 1-year-old juvenile cod influence habitat use and predator avoidance behaviour by these juvenile fish.

Materials and methods

Experimental animals

Juvenile Atlantic cod, age 0+ (i.e., <1 year, 52–82 mm standard length, SL) and 1+ (i.e., 1-year-old fish, 102–135 mm SL), and larger age 3+ cod (250–400 mm SL) were collected from Goose Cove, Trinity Bay, Newfoundland, Canada (47°51′00″N, 53°54′09″W). Collections were made during the fall of 1993 using a beach seine. Age classification was based on length frequency distributions of the fish collected by us, and by other researchers working in Trinity Bay (D.A. Methven, Memorial University of Newfoundland). Our age classification is also consistent with that reported in other studies (Keats et al. 1987; Methven and Bajdik 1994). Fish were transported to the laboratory where the different age groups were housed in separate flow-through tanks (1 × 1 × 0.47 m deep). Each tank was supplied with a mixture of ambient and heated seawater so as to maintain temperatures at 5–10°C. Cod were fed a diet of minced or chopped capelin (*Mallotus villosus*).

Experimental arena

Substrate selection experiments were conducted in two large flow-through tanks (2 × 2 × 0.5 m deep) (Fig. 1) that were isolated in a room to eliminate outside disturbances. All observations were made from behind a blind while peering through a small viewing slit. The experimental tanks were maintained at a water temperature of 5–10°C, to match that of the holding tanks. Illumination was provided by four 15-W incandescent bulbs suspended 1.17 m above the water's surface on a 0.9 × 0.9 m frame that was centred over the experimental tank. The lighting regime consisted of a programmed 12 h L : 12 h D cycle. Water depth in the tank was maintained at 0.45 m.

The eight larger cod (age 3+, hereafter called predators) used in this experiment were housed one in each of four predator chambers built into each corner of each experimental tank (Fig. 1). Each predator chamber was separated from the remainder of the tank by an opaque plexiglass partition (1 × 0.5 m high). A sliding door (15 × 19 cm high) was built into the centre of each partition and could be opened by the observer from behind the blind. When opened, the sliding door provided the only means by which a predator could enter the experimental arena.

Mineral substrates tested in this experiment were combinations of sand and gravel (SG) or sand and cobble (SC). Each substrate in a pair covered one half of the bottom of the experimental arena (Fig. 1), and the location of each substrate was randomly assigned. Substrates were collected from a local beach, cleaned, and sorted according to particle size (substrate types) using a series of metal Tyler sieves: sand (<1 mm diameter), gravel (4–16 mm diameter), and cobble (70–200 mm diameter). These substrates, which are found in the inshore environment of Newfoundland, represent substrates that do (cobble) and do not (sand and gravel) provide refuge from predation for age 0+ cod (Gotceitas and Brown 1993). We did not test a combination of gravel and cobble, as earlier experiments demonstrated no significant difference in the use of sand and gravel when these were paired with cobble (Gotceitas and Brown 1993).

Experimental design

Predators were trained to enter the experimental arena once the door to their chamber was opened (see Gotceitas and Brown 1993). Once training was complete, predators were fed on a weekly basis to keep them hungry.

Each combination of substrates was tested five times and was presented to groups (n = 5 juveniles/group) of experimentally naive age 0+ and 1+ cod. The basic design of the experiment was first to monitor substrate use by age 0+ or 1+ juveniles before, during, and following exposure to a predator, and then to repeat these observations with the other age-class of juveniles present.

Each experimental trial lasted 4 days. The evening prior to a trial (16:30), five age 0+ or 1+ cod were introduced into the experimental tank to allow sufficient time for the juveniles to acclimate to their new surroundings. On day 1 of each trial, the number of juvenile fish on each of the two substrates was recorded at 1-min intervals during three 15-min observation sessions conducted at 09:00, 12:00, and 16:00. This was repeated on day 2 for the observation periods at

09:00 and 12:00. During these five sessions, the juveniles were not exposed to a predator ('before predator,' BP).

Immediately following the session at 12:00 on day 2, an arbitrarily selected predator was released into the experimental arena and the distribution of both predator and juvenile fish was monitored for the next 15 min. The predator remained in the experimental arena for a subsequent 30 min, at the end of which another 15-min observation session was conducted. These sessions were referred to as 'with predator' (WP) treatment. The experimental arena was monitored during the entire hour that the predator was present, to determine when the first prey was caught, and the number of juvenile cod captured. Following the second 15-min observation session, the predator was returned to its chamber.

A final 15-min observation session was conducted at 16:00 on the 2nd day of the trial to examine substrate use by juvenile cod following exposure to a predation threat ('after predator,' AP).

On the morning of day 3 (08:30), five individuals of the other age-class of juvenile cod were introduced into the experimental arena. At the same time, the juvenile cod remaining in the tank from days 1 and 2 were counted, and when necessary, additional fish of the original age-class were added to maintain a count of five fish of each age-class. This marked the start of the interaction portion of the experimental trial. Thirty minutes after the introduction of the other age-class of fish, the number of fish from each age-class (i.e., 0+ and 1+) present on each of the two substrates was recorded at 1-min intervals during the first of three 15-min observation periods, conducted as on day 1 of the trial. Both age-classes were again observed for an additional two sessions at 09:00 and 12:00 the following day (day 4). All of these observations were in the absence of a predator (BP).

Because of the difference in size between the two age-classes of juvenile cod, there was the possibility that the larger, age 1+ fish could attack and consume the smaller age 0+ fish. Therefore, such attacks were also monitored and recorded during observation sessions.

Immediately following the 12:00 session on day 4, a predator was introduced into the experimental arena and the distribution of the predator, age 0+ cod, and age 1+ cod was monitored for the next 15 min. As described earlier for the predator session of day 2, a second observation session was conducted 45 min after the initial introduction of the predator. Again, the experimental arena was monitored during the entire WP period to determine when the first prey of each age-class was caught, and the number of juveniles of each age-class captured. Following the final observation session, the predator was removed from the arena and returned to its chamber.

A final 15-min observation session (AP) was conducted at 16:00 on the final day of the trial as a means of examining any effects earlier exposure to a predator may have on substrate use by juvenile cod.

Statistical analysis

To analyze the proportion of time juvenile cod spent on each available substrate, data were converted as follows. For each substrate, we first summed the number of fish counted on that substrate at every 1-min interval (max. 5 fish) over the 15-min observation session (max. 5 fish × 15 1-min intervals = 75). The total for each substrate was then divided by the sum of the number of fish present in the experimental arena (i.e., total number regardless of substrate association) at every 1-min interval (max. 5 fish) over the 15-min observation session (max. 1 or 100% of time on any one substrate; 75 for any one substrate / 75 for the experimental arena). By doing this, we could account for the fact that juveniles may have been lost to predation and therefore deal with the proportion of time the surviving fish spent over each mineral substrate offered within each combination.

To answer our questions regarding substrate use by the different age-classes of juvenile cod, both when each age-class was alone in the experimental tank and when it was part of a mixed group, and to determine if the juvenile's substrate use was affected by the presence or absence of a predator, we performed a multivariate analysis of variance (MANOVA) on the proportion data (GLM procedure, SAS Institute Inc. 1988). Our MANOVA model statement considered the following variables: (*i*) the proportion of time juveniles of each age-class spent on each available substrate during each of the predator treatments (i.e., BP, WP, AP), (*ii*) the age of the juvenile cod (i.e., 0+ or 1+), (*iii*) whether this age-class was alone in the tank or with the other age-class present, (*iv*) days of each experimental trial, (*v*) all observation sessions made for each predator treatment throughout the course of each 4-day trial, and finally (*vi*) combination of available substrates.

From the MANOVA analysis, least square means tables were generated for both fine (i.e., sand) and coarse (i.e., gravel or cobble) substrates, which enabled us to examine more closely how substrate use by juvenile cod was affected by exposure to a predator when alone and in the presence of the other age-class of juveniles. Chi-square analysis was used to compare substrate use by the fish within each predator treatment (i.e., test for substrate preference).

Chi-square analysis was also used to examine the predator's use of available substrates (for each combination of substrates) when there was a single age-class of juveniles present in the experimental arena versus both age 0+ and 1+ juveniles present. This enabled us to determine if the predator responded differently (with respect to substrate preference) towards the different age-classes of juveniles, and whether or not the presence of a larger mixed group had any influence on substrate use by the age 3+ predator.

We examined the number of juvenile cod captured and the latency until the first juvenile was captured, by an ANOVA. Data were $\log_{10}$ transformed to achieve normality. The calculated least square means tables enabled us to compare the numbers of juveniles captured and latency until first capture, between substrate combinations, within age-classes, and to determine whether the presence of conspecifics provided any protection from predation. Results from the least square means tables were compared with a corrected Bonferroni p value of 0.002.

Unless otherwise stated, the level of significance was set at 0.05 for all statistical analyses.

Results

Initial MANOVA results showed nonsignificant effects on substrate use by the juvenile cod with respect to both day and session effects, for both substrate combinations (Hotelling-Lawley trace statistic for SG: day, $F_{[8,284]} = 0.47$, $p = 0.88$; session, $F_{[40,252]} = 0.31$, $p = 1$; and SC: day, $F_{[8,284]} = 0.74$, $p = 0.66$; session, $F_{[40,252]} = 1.11$, $p = 0.30$). In contrast, substrate use by juvenile cod was affected by substrate combination ($F_{[2,295]} = 13.43$, $p = 0.0001$), their age-class ($F_{[4,588]} = 6.55$, $p = 0.0001$), the presence or absence of the other age-class of juveniles ($F_{[8,588]} = 9.71$, $p = 0.0001$), and whether or not there was a predator present ($F_{[32,588]} = 9.66$, $p = 0.0001$).

Single age-class of juvenile cod

Within each combination of substrates, and when the age-classes were alone, substrate use by age 0+ and 1+ cod was similar (Fig. 2). The pattern of SG use by age 0+ and 1+ cod was consistent among the three predator treatments (Table 1), and both age-classes preferred sand over gravel (χ^2, Table 2, Fig. 2). Use of SC by juvenile cod was similar before and after exposure to a predator, but changed with a predator present (Table 1). Again, juveniles preferred sand before and after exposure to a predator, but actively sought refuge in the interstitial spaces of the cobbles when an age 3+ cod was present

Table 1. Chi-square analyses comparing use of sand and gravel (SG) and sand and cobble (SC) by age 0+ and 1+ cod within each predator treatment (i.e., before (BP), with (WP), and after exposure (AP)), when each age-class of juvenile was the only age-class present (alone) and when the other age-class of juvenile was also present (mixed).

Substrate	Age-class	Predator treatment	χ^2 alone vs. mixed	Preference		
				Age(s) present	χ^2	Substrate
SG	0+	BP	51.2*	Alone	24.2*	S>G
				Mixed	27.0*	G>S
		WP	129.2*	Alone	25.8*	S>G
				Mixed	125.8*	G>S
		AP	39*	Alone	17.0*	S>G
				Mixed	22.4*	G>S
	1+	BP	0.0368	Alone and mixed	309*	S>G
		WP	42.7*	Alone	55.0*	S>G
				Mixed	3.06	S=G
		AP	12.3*	Alone	8.7*	S>G
				Mixed	60.8*	S>G
SG	0+	BP	394*	Alone	282*	S>C
				Mixed	123.4*	C>S
		WP	18.2*	Alone	148*	C>S
				Mixed	356*	C>S
		Ap	91.9*	Alone	29.4*	S>C
				Mixed	65*	C>S
	1+	BP	43.06*	Alone	361.8*	S>C
				Mixed	108*	S>C
		WP	18.05*	Alone	379*	C>S
				Mixed	549.6*	C>S
		AP	0.858	Alone and mixed	16.8*	S>C

Note: $x > y$ indicates that substrate x was preferred to substrate y. S, sand; G, gravel; C, cobble.
*Significant at $\chi^2_{0.95} = 3.841$ (df = 1).

(Table 1, Fig. 2). When cobble was unavailable, we observed that juvenile cod kept a maximum distance between themselves and the predator and reduced their overall activity.

Both age-classes of juvenile cod present

The presence of younger conspecifics had no influence on substrate use by age 1+ cod. This was true for both combinations SG and SC (Fig. 2). In both combinations, sand was the preferred substrate (Table 1, Fig. 2) before and after exposure to a predator (Table 2).

In contrast to the age 1+ cod, substrate use by age 0+ fish changed significantly in both combinations SG and SC when age 1+ fish were present as compared with that observed when the age 0+ juveniles were alone (Fig. 2).

With SG, gravel was the preferred substrate by the age 0+ fish when age 1+ cod were present, regardless of predator treatment (Table 1, Fig. 2). This preference did not change between predator treatments (Table 2).

When age 1+ fish were also in the SC tank with the age 0+ cod, cobble was the preferred substrate before, with, and after exposure to a predator (Table 1, Fig. 2). However, although cobble was the preferred substrate of the age 0+ cod under all three predator treatments when in the presence of age 1+ cod, its use increased when a predator was also introduced (Table 2).

In both combinations SG and SC, age 0+ cod preferred sand in the absence of age 1+ fish, but switched to the coarse substrate when age 1+ cod were present (Fig. 2). Given that when alone, both age 0+ and 1+ cod showed a similar pattern of substrate use, this switch by the age 0+ fish when in the presence of age 1+ conspecifics indicates that the age 0+ fish were avoiding the age 1+ fish. This avoidance probably constitutes predator avoidance by the age 0+ juveniles, as age 1+ fish did capture some of the age 0+ cod (nine incidents of cannibalism).

Predator

Substrate use

Regardless of the age-class of juvenile cod present in the experimental tank, there was no consistent pattern of substrate preference by the predator for either the fine or coarse substrate in SG (Table 3, Fig. 3). In contrast, the predator exhibited a significant preference for sand in SC (Table 3, Fig. 3).

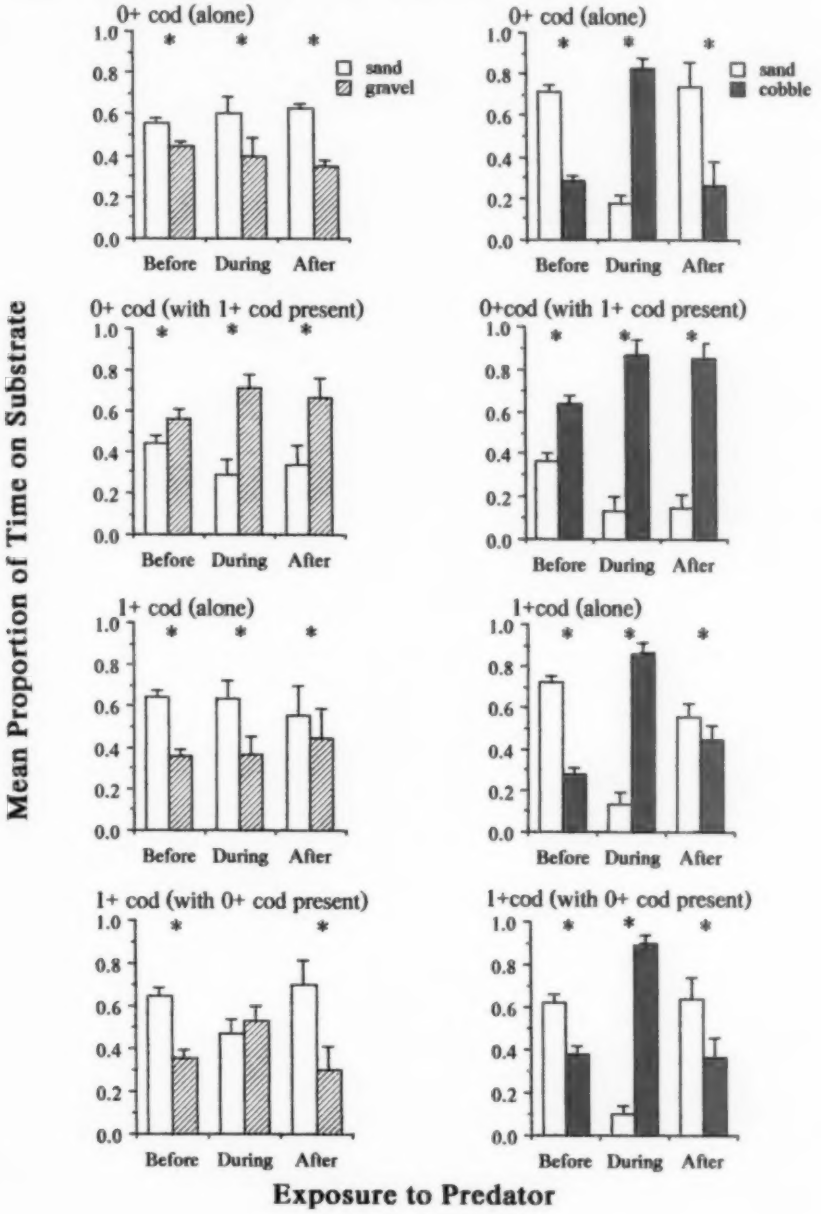
In all cases, the juveniles exhibited a preference for the substrate least preferred by the predator (Fig. 3), indicating that the juveniles were actively avoiding the predator's location in the experimental arena.

Predation on juvenile cod

Number captured

Neither the combination of substrates nor whether one or two age-classes of juvenile cod were present affected the number of juvenile cod captured by the predator (ANOVA; $F_{[1,33]} = 0.95$, $p = 0.3372$; and $F_{[2,33]} = 1.67$, $p = 0.1798$, respectively).

Fig. 2. Mean (+1 SE) proportion of time age 0+ and 1+ cod spent on sand and gravel (SG) and sand and cobble (SC) before, during, and after exposure to a predator, when in the absence or presence of the other age-class. Within each predator condition (i.e., BP, WP, AP), * indicates a significant preference between the two substrates by the juvenile cod.



The age of the juveniles was the only factor that influenced the number captured by the predator ($F_{[2,33]} = 10.78$, $p = 0.0003$). Least square means comparisons generated from these data revealed that the only significant difference was that more age 0+ fish were captured than age 1+ cod, when comparing the number of juveniles captured when each age-class was the only one present in the experimental arena and offered a choice of sand and gravel ($t = 3.73$, $p = 0.0007$) (Fig. 4A).

Latency

Substrate combination ($F_{[1,33]} = 5.18$, $p = 0.0294$), age-class of juvenile cod ($F_{[2,33]} = 22.69$, $p = 0.0001$), and whether one or

Table 2. Least square means analysis, t and p values, comparing the use of fine (sand) and coarse (gravel or cobble) substrate by juvenile cod in combinations SG (sand and gravel) and SC (sand and cobble) between predator treatments (before (BP), with (WP), and after (AP) exposure).

		BP vs. WP		BP vs. AP		WP vs. AP	
Age-class		t	p	t	p	t	p
		Fine substrate					
0+ alone	SG	0.65	0.52	0.77	0.44	0.25	0.80
	SC	7.67	0.0001*	0.22	0.83	0.54	0.0001*
0+ mixed	SG	2.05	0.041	1.11	0.26	0.40	0.69
	SC	4.09	0.0001*	1.06	0.29	1.84	0.067
1+ alone	SG	0.15	0.88	0.93	0.35	0.73	0.467
	SC	8.16	0.0001*	1.80	0.073	3.97	0.0001*
1+ mixed	SG	2.48	0.014	0.61	0.54	2.24	0.026
	SC	7.73	0.0001*	0.31	0.76	5.0	0.0001*
		Coarse substrate					
0+ alone	SG	0.65	0.52	0.99	0.33	0.44	0.66
	SC	7.67	0.0001*	0.22	0.83	5.43	0.0001*
0+ mixed	SG	2.05	0.041	1.12	0.26	0.40	0.69
	SC	4.08	0.0001*	1.06	0.29	1.84	0.067
1+ alone	SG	0.15	0.88	0.93	0.35	0.73	0.47
	SC	8.16	0.0001*	1.80	0.073	3.97	0.0001*
1+ mixed	SG	2.50	0.014	0.61	0.54	2.24	0.026
	SC	7.71	0.0001*	0.31	0.76	4.99	0.0001*

* Significant difference between predator treatments compared with the corrected Bonferroni p value of 0.002.

Table 3. Chi-square analyses comparing use of available substrates by juvenile cod when in the tank as a solitary age-class of juveniles (alone) and when both age-classes of juveniles were present (mixed).

			Preference		
Substrate	Age-class	χ^2 alone vs. mixed	Age(s) present	χ^2	Substrate
Sand and gravel	0+	25.9*	Alone	7.7*	G>S
			Mixed	19.4*	S>G
	1+	11.53*	Alone	0.107	S=G
			Mixed	19.44*	S>G
Sand and cobble	0+	2.27	Alone and mixed	38.5*	S>C
	1+	0.017	Alone and mixed	60*	S>C

Note: $x > y$ indicates that substrate x was preferred to substrate y. S, sand; G, gravel; C, cobble.
*Significant at $\chi^2_{0.95} = 3.841$ (df = 1).

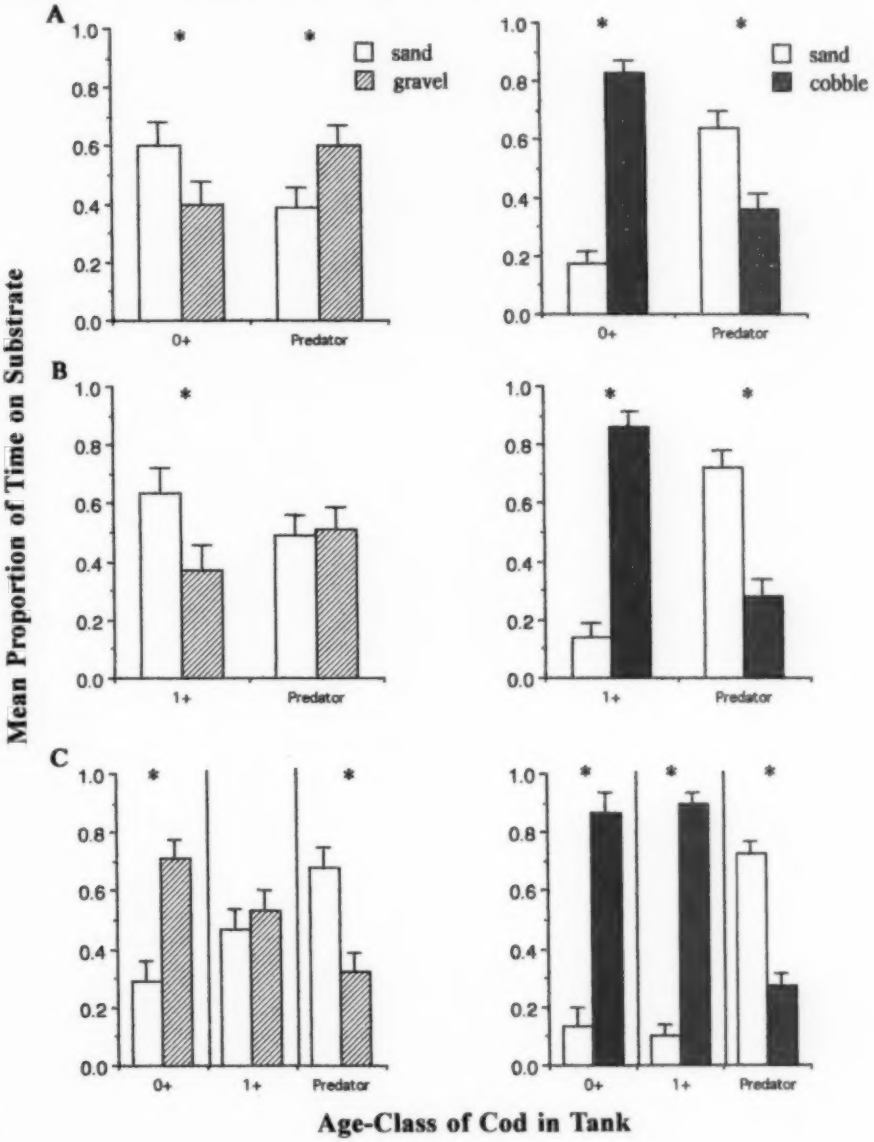
both age-classes were present ($F_{[4,33]} = 8.46$, $p = 0.0001$) all influenced the time it took a predator to capture its first juvenile. With sand and gravel present, it took the predator longer to capture an age 1+ cod than an age 0+ fish ($t = 7.08$, $p = 0.0001$) (Fig. 4B); however, when cobble was present, capture times did not differ ($t = 0.85$, $p = 0.40$). It took a predator longer to capture an age 0+ cod with cobble present when this was the only age-class present ($t = 6.17$, $p = 0.0001$) (Fig. 4B). In substrate combination SG, it took the predator longer to capture an age 0+ fish when age 1+ fish were present ($t = 5.61$, $p = 0.0001$) (Fig. 4B).

Discussion

Our results demonstrate that bottom substrate type, threat of predation, and the presence of conspecifics all influence habitat use by juvenile cod. When tested individually, age 0+ and 1+ cod showed a similar pattern of substrate use and responded in a similar manner to the threat of predation. In the absence of a predator both age 0+ and 1+ cod preferred fine-grained substrate. In contrast, in the presence of a predator, both age-classes either hid in cobble, or, if this substrate was not present, kept a maximum distance from the predator. These results are consistent with those of Gotceitas and Brown (1993) for age 0+ cod.

Various studies have demonstrated an increased use of structurally complex habitat by a variety of fish species when exposed to the threat of predation (Orth et al. 1984; Tabor and Wurtsbaugh 1991). Association with these complex habitats reduces predation risk (Gotceitas and Colgan 1989; Gotceitas and Brown 1993). In the wild, juvenile cod associate with structurally complex habitats and this association has been attributed to seeking cover from predation (Keats et al. 1987; Lough et al. 1987; Tupper and Boutilier 1995). In our study,

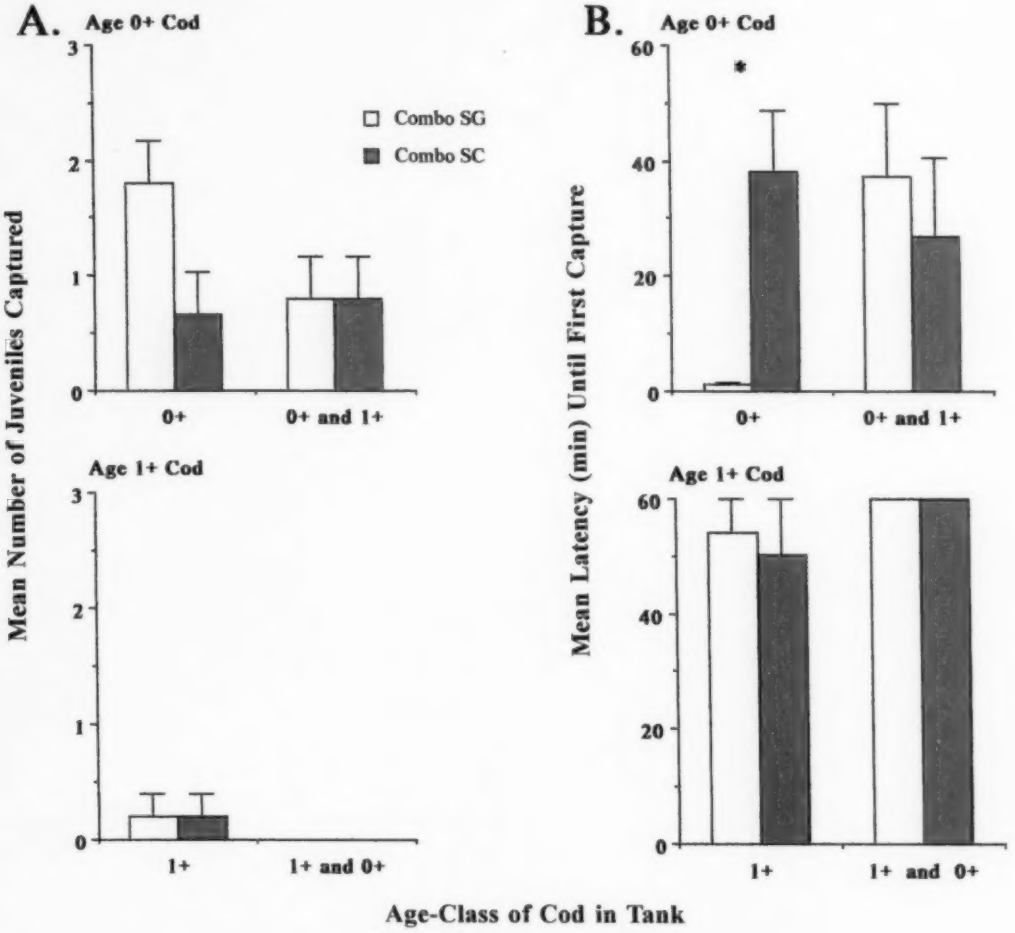
Fig. 3. Mean (+1 SE) proportion of time juvenile cod spent on available substrates when in the presence of a predator. Juvenile cod of (A) age 0+ alone, (B) age 1+ alone, and (C) both age 0+ and 1+ were present in the experimental tank. The substrate combinations were sand and gravel, and sand and cobble. * indicates a significant preference between the two substrates.



use of the cobble substrate significantly reduced the risk of predation to juvenile cod. Therefore, our results support the suggestion from these field studies that juvenile cod associate with structurally complex habitats as a means of reducing their risk of predation. Further, selection for cobble by both age-classes when exposed to a potential predator indicates an ability by juvenile cod to identify predation risk and respond in a manner that increases their chances of survival.

In the field, age 0+ and 1+ cod have been captured in the same sample (Riley and Parnell 1984; Gjosæter 1987; Keats et al. 1987; Methven and Bajdik 1994; S. Fraser, V. Gotceitas, and J.A. Brown, personal observations), suggesting that these two age-classes of juveniles coexist in the same habitat. These field results are consistent with our findings that substrate selection by each of these two age-classes of juvenile cod is virtually identical. However, although the presence of age 0+ cod did not alter the pattern of substrate use by age 1+ cod, substrate use by age 0+ individuals was significantly

Fig. 4. (A) Mean (+1 SE) number of prey captured by the predator during the 60 min it had access to the fish in the experimental arena, and (B) mean (+1 SE) latency (min) until the predator captured its first prey, with respect to the combination of substrates present (SG and SC), the age of the juveniles (age 0+ and 1+), and the presence or absence of the other age-class of juvenile.



influenced by the presence of age 1+ conspecifics; age 0+ cod avoided substrates occupied by age 1+ cod, thus minimizing their contact with these older fish. Contrary to our results on substrate use by each age-class individually, results from trials where both age-classes were tested simultaneously suggest that age 0+ and 1+ cod are unlikely to coexist in the same habitat. Samples taken in the field typically cover a relatively large spatial and (or) temporal scale, and coexistence between age 0+ and 1+ cod may well occur on this larger scale. However, our results suggest that a certain level of segregation in habitat use and distribution between age 0+ and 1+ cod could be expected on a smaller spatial scale.

In nature, populations typically consist of heterogeneous sizes or stages of individuals, which often leads to overlap between generations (Wissinger 1992). In turn, such overlap can lead to interspecific or intraspecific competition and predation (Wissinger 1992). For example, there is often a segregation of a size-structured guild with respect to habitat use by different year-classes (Sweitzer and Berger 1992; Wissinger 1992). As discussed above, because age 0+ and 1+ juvenile cod preferred the same substrate types when alone in the tank, and age 0+ cod avoided older juveniles, we would expect there to be some level of habitat segregation between different year-classes in wild populations. This does seem to be the case when considering juveniles and adults: in Newfoundland, juveniles appear to occupy predominantly nearshore habitats whereas the larger and older fish migrate between the offshore and inshore (Lilly 1987). Similar segregation of juveniles and adults by habitat has been noted in other species of fish and macroinvertebrates (e.g., Kneib 1987). This large-scale habitat segregation between juvenile and adult cod may reflect predator avoidance on the part of the juveniles, as adult cod are known to feed on younger conspecifics (Gjosæter 1987; Bogstad et al. 1994; present study). Alternatively, habitat segregation between juvenile and adult cod could be the result of age- or size-specific changes in the risk of predation to interspecific

predators (Riley and Parnell 1984; Sweitzer and Berger 1992) and (or) required resources (Scott 1982; Nordeide and Fosså 1992; van Duren and Glass 1992).

Our findings that age 0+ cod tend to avoid age 1+ conspecifics indicate how certain age-classes may restrict habitat use by other age-classes in the wild. For example, when pelagic age 0+ juvenile cod migrate inshore (Riley and Parnell 1984) and attempt to settle in habitats containing preferred bottom types, our results suggest that if age 1+ conspecifics already occupy these areas, the age 0+ fish would be expected to avoid settlement in those areas. Because the survival of postsettlement age 0+ cod is at least partially dependent on habitat (Lough et al. 1987; Gotceitas and Brown 1993), a direct result of the intraspecific interactions between age-classes of juvenile cod demonstrated in our study could be reduced survival of the 0+ age-class if preferred (i.e., safe) habitat is limited (e.g., increased predation in unsafe habitats). In addition to affecting the level of natural recruitment, intraspecific constraints on habitat use would also be expected to influence the success of enhancement programs involving the introduction of cod into a system (Nordeide and Fosså 1992; Smedstad et al. 1994).

Overall, our results suggest that the presence of age 1+ cod has a negative impact on age 0+ fish by restricting their use of habitats. However, we also found evidence that this avoidance of older conspecifics can break down under certain conditions, and that the presence of age 1+ cod can even provide certain advantages to age 0+ fish. For example, with no safe habitat, significantly fewer age 0+ cod were captured by the predator and it took the predator significantly longer to capture an age 0+ cod when age 1+ conspecifics were also present. These results suggest that under certain conditions, an association with age 1+ conspecifics may be beneficial to age 0+ cod. In our study, this increased protection from predation when in the presence of age 1+ fish may have been the result of an increased number of prey and (or) the size composition of prey present. While further study is needed to identify the specific cause of this reduced mortality to age 0+ cod, similar protection from predation as a result of group size and group structure has been documented for other fish species, as well as other taxa (Lima and Dill 1990).

When cobble was available to the juvenile cod as a possible refuge, age 0+ cod hid in this coarse substrate along with the older juveniles when confronted with the risk of predation. Therefore, under conditions of mutual danger, intraspecific interactions between age 0+ and 1+ cod seemingly disappeared. Taken together, our results suggest that the influence of intraspecific interactions on the distribution and survival of age 0+ and 1+ cod is complex in nature.

In summary, our laboratory experiments provide preliminary information regarding the effects of intraspecific interactions on age-class specific habitat use and risk of predation to age 0+ and 1+ juvenile Atlantic cod. Our results also offer a possible explanation for the fluctuations in survival observed in Atlantic cod. For example, if suitable habitat is limited and the presence of age 1+ cod in this habitat keeps age 0+ fish from settling there, the number of age 0+ fish surviving to age 1+ may be dramatically reduced. In turn, this may translate into a poor year-class for the fishery, suggesting that the age 0+ stage in the life history of Atlantic cod may represent a bottleneck to recruitment.

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References

Anderson, T.W. 1994. Role of marcoalgal structure in the distribution and abundance of a temperate reef fish. Mar. Ecol. Prog. Ser. **113**: 279–290.

Bogstad, B., Lilly, G.R., Mehl, S., Palsson, O.K., and Stefansson, G. 1994. Cannibalism and year-class strength in Atlantic cod (*Gadus morhua*) in Arcto-boreal ecosystems (Barents Sea, Iceland and eastern Newfoundland). ICES Mar. Sci. Symp. **198**: 576–599.

Carr, M.H. 1994. Effects of macroalgal dynamics on recruitment of a temperate reef fish. Ecology, **75**: 1320–1333.

Gjosæter, J. 1987. Habitat selection and inter year class interaction of young cod (*Gadus morhua*) in aquaria. Flodevigen raportser, Arendal, Norway. ISSN 0333-2594. pp. 27–36.

Gotceitas, V., and Brown, J.A. 1993. Substrate selection by juvenile cod (*Gadus morhua*): effects of predation risk. Oecologia, **93**: 31–37.

Gotceitas, V., and Colgan, P. 1989. Predator foraging success and habitat complexity: quantitative test of the threshold hypothesis. Oecologia, **80**: 158–166.

Keats, D.W., Steele, D.H., and South, G.R. 1987. The role of fleshy macroalgae in the ecology of juvenile cod (*Gadus morhua* L.) in inshore waters off eastern Newfoundland. Can. J. Zool. **65**: 49–53.

Kneib, R.T. 1987. Predation risk and use of intertidal habitats by young fishes and shrimp. Ecology, **68**: 379–386.

Lilly, G.R. 1987. Interactions between Atlantic cod (*Gadus morhua*) and capelin (*Mallotus villosus*) off Labrador and eastern Newfoundland: a review. Can. Tech. Rep. Fish. Aquat. Sci. No. 1567.

Lima, S.L., and Dill, L.M. 1990. Behaviourial decisions made under the risk of predation: a review and prospectus. Can. J. Zool. **68**: 619–640.

Lough, R.G., Valentine, P.C., Potter, D.C., Auditore, P.J., Bolz, G.R., Neilson, J.D., and Perry, R.P. 1987. Ecology and distribution of juvenile cod and haddock in relation to sediment type and bottom currents on eastern Georges Bank. Mar. Ecol. Prog. Ser. **56**: 1–12.

Methven, D.A., and Bajdik, C. 1994. Temporal variation in size and abundance of juvenile Atlantic cod (*Gadus morhua*) at an inshore site off eastern Newfoundland. Can. J. Fish. Aquat. Sci. **51**: 78–90.

Nordeide, J.T., and Fosså, J.H. 1992. Diet overlap between two subsequent year-classes of juvenile coastal cod (*Gadus morhua*) and wild and reared cod. Sarsia, **77**: 111–117.

Orth, R.J., Heck, K.L., and van Montfrans, J. 1984. Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator–prey relationships. Estuaries, **7**: 339–350.

Riley, J.D., and Parnell, W.G. 1984. The distribution of young cod. *In* The propagation of cod *Gadus morhua* L. *Edited by* E. Dahl, D.S. Danielssen, E. Moksness, and P. Solemdal. Flodevigen raportser, Arendal, Norway. ISSN 0333-2594. pp. 563–580.

SAS Institute 1988. SAS/STAT user's guide, release 6.03 edition. SAS Institute Inc., Cary, N.C.

Scott, J.S. 1982. Selection of bottom type by groundfishes of the Scotian Shelf. Can. J. Fish. Aquat. Sci. **39**: 943–947.

Smedstad, O.M., Salvanes, A.G.V., Fosså, J.H., and Nordeide, J.T. 1994. Enhancement of cod, *Gadus morhua* L., in Masfjorden: an overview. Aquacult. Fish. Manage. **25**(Suppl. 1): 117–128.

Sweitzer, R.A., and Berger, J. 1992. Size-related effects of predation on habitat use and behaviour of porcupines (*Erethizon dorsatum*). Ecology, **73**: 867–875.

Tabor, R.A., and Wurtsbaugh, W.A. 1991. Predation risk and the importance of cover for juvenile rainbow trout in lentic systems. Trans. Am. Fish. Soc. **120**: 728–738.

Tupper, M., and Boutilier, R.G. 1995. Size and priority at settlement determine growth and competitive success of newly settled Atlantic cod. Mar. Ecol. Prog. Ser. **118**: 295–300.

van Duren, L.A., and Glass, C.W. 1992. Choosing where to feed: the influence of competition on feeding behaviour of cod, *Gadus morhua* L. J. Fish Biol. **41**: 463–471.

Wigley, S.E., and Serchuck, F.M. 1992. Spatial and temporal distribution of juvenile Atlantic cod *Gadus morhua* in the Georges Bank – Southern New England region. Fish. Bull. **90**: 599–606.

Wissinger, S. 1992. Niche overlap and the potential for competition and intraguild predation between size-structured populations. Ecology, **73**: 1431–1444.

Size structure and species composition of plankton communities in deep Ontario lakes with and without *Mysis relicta* and planktivorous fish

M. Jane R. Almond, Ellen Bentzen, and William D. Taylor

Abstract: Twenty-three deep oligotrophic lakes in the Huntsville–Bancroft region of Ontario and three locations in Lake Ontario were sampled to investigate the impact of size-selective planktivores on the size structure and species composition of plankton communities. The planktivores include *Mysis relicta* and planktivorous fishes, usually lake herring (*Coregonus artedii*) in the Huntsville–Bancroft region or alewife (*Alosa pseudoharengus*) in Lake Ontario. Previous work had related the presence of these planktivores to higher contaminant levels in lake trout (*Salvelinus namaycush*). We found that lakes with planktivorous fish had a lower proportion of biomass contributed by zooplankton over 1 mm than lakes without *M. relicta* and planktivorous fish, which suggests a direct effect of these visual planktivores on larger zooplankton. Lakes with both *M. relicta* and planktivorous fish had a lower biomass of both small and large *Daphnia* spp. than lakes with planktivorous fish only. This suggests that the effect of *M. relicta* extends to smaller cladocerans than those affected by planktivorous fish. Planktivory did not have a strong effect on the species composition of plankton. On the basis of the filter fractionation of particulate phosphorus, lakes with planktivores had a smaller proportion of large particles than lakes without planktivores.

Résumé : On a échantillonné vingt-trois lacs oligotrophes profonds de la région de Huntsville–Bancroft, en Ontario, et trois sites du lac Ontario pour étudier l'impact des planctivores consommant sélectivement certaines tailles sur la structure des tailles et de la composition spécifique des communautés de plancton. Ces planctivores sont notamment *Mysis relicta* et des poissons planctivores, habituellement le cisco de lac (*Coregonus artedii*) dans la région de Huntsville–Bancroft ou le gaspareau (*Alosa pseudoharengus*) dans le lac Ontario. Des travaux antérieurs ont établi un rapport entre la présence de ces planctivores et les teneurs en contaminants plus élevées chez le touladi (*Salvelinus namaycush*). Nous avons constaté que, par rapport aux lacs sans *M. relicta* et sans poissons planctivores, les lacs à poissons planctivores abritaient une proportion inférieure de biomasse provenant des espèces de zooplancton de plus de 1 mm, ce qui suggère un effet direct de ces planctivores visibles sur les espèces plus grandes de zooplancton. Les lacs contenant des *M. relicta* et des poissons planctivores avaient une plus faible biomasse tant pour les petites que pour les grandes daphnies par rapport aux lacs ne contenant que des poissons planctivores. Ceci suggère que l'effet des *M. relicta* s'étend aux espèces de cladocères de taille plus petite que celles consommées par les poissons planctivores. D'après des mesures de fractionnement sur filtre du phosphore particulaire, on a constaté que les lacs à populations de planctivores présentaient une plus faible proportion de grandes particules que les lacs sans planctivores.
[Traduit par la Rédaction]

Introduction

Selective predation by visual planktivores can change the size structure and species composition of freshwater zooplankton communities. In general, the largest, most visible zooplankters are consumed by size-selective planktivores (Brooks and Dodson 1965; Zaret 1980). Abundant visual planktivores are associated with lower biomass and smaller mean size of zooplankton, including smaller mean size of individuals within a species. These effects on zooplankton may, in turn, affect the abundance of smaller planktonic organisms and, overall, the size distribution and biomass of plankton. For example, in enclosures with planktivorous fish, there is a decrease in the abundance of mesoplankton and an increase in the abundance of smaller particles, particularly nanoplankton (Mazumder et al. 1988; Vanni 1990; Drenner and Smith 1991).

In most of the deep glacial lakes belonging to the St. Lawrence system, lake trout (*Salvelinus namaycush*) is the top piscivore in the pelagic zone (Kerr and Martin 1970; Trippel and Beamish 1989). The abundance and species composition of planktivores, however, varies among the lakes because of uneven postglacial dispersion and recent introductions. In some lakes, lake herring (*Coregonus artedii*), lake whitefish (*Coregonus clupeaformis*), rainbow smelt (*Osmerus mordax*), alewives (*Alosa pseudoharengus*), and opossum shrimp (*Mysis relicta*) form an intermediate trophic level between lake trout and zooplankton. However, in other lakes, some or

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Table 1. Characteristics of lakes sampled in the Huntsville–Bancroft region of Ontario.

Lake	Abbreviation	Latitude (°N)	Longitude (°W)	Surface area (ha)	Mean depth (m)	Max. depth (m)	*Mysis relicta*	Lake herring (*Coregonus artedii*)	Lake whitefish (*Coregonus clupeaformis*)	Rainbow smelt (*Osmerus mordax*)
***Mysis relicta* and planktivorous fish absent**										
Anstruther	Anstr	44°44′16″	78°13′21″	634	12.7	37.0	—	—	—	—
Eels	Eels	44°53′31″	78°07′20″	946	6.8	30.1	—	—	—	—
Kawagama	Kawag	45°18′03″	78°44′08″	2800	21.5	70.0	—	—	—	—
Kennisis	Kenn	45°13′03″	78°37′47″	1417	23.5	70.0	—	—	—	—
Silent	Silent	44°54′44″	78°03′39″	115	6.2	23.2	—	—	—	—
Planktivorous fish present										
Chandos	Chand	44°49′50″	77°58′45″	1733	13.2	48.0	—	Y	—	—
Haliburton	Halib	45°12′35″	78°24′39″	1014	17.6	55.4	—	Y	Y	—
Halls	Halls	45°06′25″	78°44′20″	540	27.2	69.0	—	—	Y	Y
Koshlong	Kosh	44°58′12″	78°29′20″	396	10.4	43.0	—	—	—	Y
Limerick	Limrk	44°53′08″	77°37′30″	734	8.3	28.0	—	—	Y	—
Little Hawk	LHawk	45°09′00″	78°42′27″	248	31.0	87.0	—	—	Y	—
Oxtongue	Oxtgue	45°22′10″	78°55′05″	246	9.9	27.0	—	Y	Y	Y
St. Nora	St Nora	45°09′32″	78°49′54″	582	15.7	40.0	—	—	Y	Y
***Mysis relicta* and planktivorous fish present**										
Boshkung	Bosh	45°03′10″	78°43′26″	690	23.4	71.7	Y	Y	Y	Y
Crystal	Crys	44°45′40″	78°28′27″	468	12.6	34.0	Y	—	Y	Y
Fairy	Fairy	45°19′36″	79°11′25″	711	21.9	74.0	Y	Y	Y	Y
Gull	Gull	44°50′18″	78°47′08″	995	16.2	39.0	Y	Y	Y	Y
Lake of Bays	Bays	45°13′28″	79°03′09″	7058	22.2	53.0	Y	Y	Y	Y
Mazinaw	Mazin	44°54′38″	77°11′53″	1590	40.5	146.0	Y	Y	—	—
Shabomeka	Shab	44°53′27″	77°08′10″	264	12.3	31.0	Y	Y	—	—
Skeleton	Skel	45°15′22″	79°26′35″	2154	30.1	62.0	Y	Y	Y	Y
Twelve Mile	TwelM	45°01′50″	78°42′12″	310	12.1	27.7	Y	Y	Y	Y
Vernon	Vern	45°19′30″	79°17′00″	1505	12.8	37.0	Y	Y	Y	Y

Note: The presence of planktivorous species is indicated by Y.

all of these planktivores are absent (Rasmussen et al. 1990; Ontario Ministry of Natural Resources, District offices, unpublished data). Because of their feeding behaviour and diet, lake herring, alewives, and *M. relicta* should have a pronounced effect on the size structure and species composition of zooplankton communities (Nero and Sprules 1986; Langeland et al. 1991; Strus and Hurley 1992). Although lake whitefish and smelt are predominately benthic feeders, young whitefish consume cladocerans and copepods (Reckahn 1970) and smelt feed in the water column as well as the lake bottom (Evans and Loftus 1987). Therefore, these species also could have an effect on the size structure and species composition of plankton communities.

Rasmussen et al. (1990) observed that lake trout from lakes with both *M. relicta* and planktivorous fish had higher levels of polychlorinated biphenyls (PCBs) than lake trout from lakes without these planktivores. Lake trout from lakes with only planktivorous fish had intermediate levels of PCBs. The higher levels of PCBs in lake trout from lakes with vertebrate and invertebrate planktivores were attributed to the longer food chain, resulting in greater bioaccumulation. Taylor et al. (1991) found that the level of hydrophobic organochlorine contaminants in net plankton samples also varied among lakes in the same area, and that contaminant level was inversely related to the biomass of plankton.

The main objective of our study was to determine whether the presence of these planktivores was associated with differences in the size structure and species composition of plankton in deep, oligotrophic lakes containing lake trout. If this is true, then differences in PCB content of lake trout might also be the result of differences in water-seston partitioning and bioaccumulation of contaminants at lower trophic levels. We also wanted to determine if continuous variables derived from the size distribution or species composition of plankton would quantify the effect of these planktivores. To produce continuous variables, we quantified the size structure of plankton greater than 0.2 μm by using the particulate phosphorus index developed by Mazumder et al. (1988). As well, we obtained the size structure and species composition of crustacean zooplankton greater than 200 μm by identifying and measuring individual zooplankton. We then produced continuous variables from this data using ordination analyses.

Materials and methods

Study sites and sampling trips

Twenty-three lakes, 18 of which were analysed by Rasmussen et al. (1990), were sampled in the Huntsville–Bancroft region of Ontario during three periods in 1992: May 12 to 24, July 12 to 23, and September 7 to 19 (Table 1). Samples were collected at about the same location on each lake during each visit. A single sampling site will be referred to as a lake, although the data obtained from each site may not necessarily reflect the lake as a whole.

Samples were also collected from Lake Ontario at stations on the north side of the lake (bottom depth 38 m), at mid-lake (178 m), and on the south shore (38 m) during three cruises of the CSS *Limnos*, from April 22 to 23, from August 5 to 13, and from October 7 to 9, 1992. The location of these stations (401, 403, and 405) is provided in Lean et al. (1987). The southern station was not sampled in April. The time periods for sampling will subsequently be referred to as spring, summer, and fall.

Total phosphorus and size distribution of particulate phosphorus

For lakes in the Huntsville–Bancroft region, temperatures were measured from the surface to 20 m at 1-m intervals down to 10 m, and at 2-m intervals from 10 to 20 m. In Lake Ontario, temperatures were measured with a continuous recording electronic bathythermograph (EBT)–transmissometer from the surface to the bottom. On the basis of the temperatures, the depths corresponding to the middle of the epilimnion and metalimnion were estimated. Ten litres of lake water was then collected from the middle of the epilimnion and the middle of the metalimnion using a Van Dorn sampler (Huntsville–Bancroft lakes) or a rosette sampler (Lake Ontario). Lake Ontario was not thermally stratified in April; however, samples were collected from 5 and 25 m at both stations 401 and 403 and from 5 and 17 m at station 405.

Two 100-mL samples of water from the epilimnion and metalimnion were put into screw-capped bottles and analysed for total phosphorus (TP) concentration. Duplicate 4-L samples of water from the epilimnion and metalimnion were filtered through a series of graded nylon screens and polycarbonate filters to fractionate the particulate matter into six size-classes: <0.2, 0.2–2, 2–20, 20–200, 200–400, and >400 μm (Mazumder et al. 1988). All screens and filters were put into clean, screw-capped test tubes; 35 mL of distilled, deionized water was added later in the laboratory. The concentration of TP in each size fraction was then determined following oxidation with potassium persulfate under pressure (Menzel and Corwin 1965) using the ascorbic acid modification of the molybdenum blue method (Strickland and Parsons 1972). Particle-size distributions were characterized by the slope parameter, obtained by regressing the proportion of particulate phosphorus (PP) retained by each filter against the log of the filter pore size (Mazumder et al. 1988). This slope parameter will be referred to as the PP slope. A PP slope of about –0.2 indicates a dominance of large particles, whereas a steeper slope of about –0.3 indicates an abundance of small particles. A slope of –0.25 indicates that the amount of PP in the four logarithmic size-classes (pico-, nano-, micro-, and meso-plankton) is similar.

Zooplankton species composition and size distribution

For lakes in the Huntsville–Bancroft region, mesoplankton was collected using a metered 0.5 m diameter net with 200-μm mesh. Vertical hauls were taken from near the bottom to the surface, with the depths of hauls ranging from 15 to 26 m. In Lake Ontario, during April, vertical hauls were taken with the same net. During August and October, a metered plankton net with a 0.30 m mouth diameter and 150-μm mesh was used. Depths of hauls in Lake Ontario ranged from 20 to 30 m. Zooplankton samples from all of the lakes were preserved in a 10% buffered formalin solution.

The presence of *M. relicta* in the lakes (Dadswell 1974) was verified in our study through net hauls and by Gordon Paterson (Trent University, personal communication). Crustacean zooplankton were identified according to Balcer et al. (1984) and Brooks (1957). Immature stages of Copepoda were categorized as calanoid copepodites and cyclopoid copepodites. Subsamples of zooplankters were counted and measured using the software ZEBRA obtained from the Ontario Ministry of the Environment. The ZEBRA program was run on a microcomputer linked to electronic calipers, a dissecting microscope, and a video camera. The biomass of individual organisms was determined on the basis of length measurements and published length–weight relationships (McCauley 1984; Sprules 1984; Culver et al. 1985; Yan and Mackie 1987) incorporated into the ZEBRA program. Biomass estimates were corrected for net efficiencies, which ranged from 36 to 97%.

Statistical analyses

All statistical analyses used Systat 5.2 (Wilkinson 1992). Differences in PP slopes among lake groups were analysed using a repeated-measures analysis of variance (RM-ANOVA). The lake groups included lakes without *M. relicta* and planktivorous fish, lakes with planktivorous fish but without *M. relicta*, and lakes with both *M. relicta* and planktivorous fish. Fisher's protected test of least significant difference (Steel and Torrie 1960) was carried out when the RM-ANOVA showed a significant ($P < 0.05$) group effect.

PP slopes were checked for normality using normal probability plots of the ANOVA residuals. The assumption of homogeneity of variance was checked using a plot of the standard deviation of PP slopes against the mean slope for each lake group. The assumptions of constant variance and constant correlation across the repeated measures factors for the RM-ANOVA were reasonably valid on the basis of the Greenhouse–Geisser and Haynh–Feld correction factors for the degrees of freedom (Winer 1971; Crowder and Hand 1990).

Missing TP values for five of the lakes sampled in September were replaced with the average TP concentration for other lakes in September of the same lake group. Four of the missing TP values were from two lakes with planktivorous fish, but without *M. relicta*, and the remaining five missing TP values were from three lakes with both *M. relicta* and planktivorous fish. Differences in TP concentrations among lake groups were then analysed using RM-ANOVA.

Principal components analysis and discriminant functions analysis were used to determine if differences in the species composition and size distribution of zooplankton among the inland lakes could be attributed to the presence or absence of planktivores. Zooplankton biomass, expressed on a volumetric basis, for both the summer and fall was included in the analyses. Principal components analysis was performed on a covariance matrix. It was not necessary to standardize the variables using a correlation matrix because the biomass variables were expressed in the same units and were transformed using natural logs. To more directly assess zooplankton biomass and size, additional derived variables were added to the discriminant functions analysis. These variables included the mean length of *Daphnia* spp., the total biomass of zooplankton, the biomass of cladocerans and copepods longer than 1 mm as a proportion of total zooplankton biomass, and the biomass of cladocerans longer than 1 mm as a proportion of total zooplankton biomass. The decision to use 1 mm as a threshold size for planktivore selectivity was based on the results of previous studies of planktivore predation. Kerr (1971) suggested that the growth efficiency of planktivorous fish is directly related to prey size. As a prey item exceeds a certain size, the energy content it contains becomes greater than the energy expended by a planktivore for its capture. Therefore, planktivores tend to consume prey items over a certain size to maximize their growth efficiency. Werner and Hall (1974) and Gardner (1981) found that bluegills (*Lepomis macrochirus*) selected *Daphnia* spp. over 1 mm. Similarly, Brooks (1968) found that marine alewives (*Alosa pseudoharengus*) preferred zooplankton over 1 mm.

Zooplankton species present in less than four of the lakes were eliminated from the analyses: *Ceriodaphnia* spp., *Eubosmina coregoni*, *Leptodora kindtii*, *Polyphemus pediculus*, *Bythotrephes cederstroemi*, *Diaphanosoma birgei*, *Onychodiaptomus sanguineus*, *Leptodiaptomus sicilis*, *Acanthocyclops vernalis*, and *Tropocyclops extensus*. *Limnocalanus macrurus* and *Epischura lacustris* were also eliminated from the analyses. *Limnocalanus macrurus* is considered to be a glacial opportunist because its distribution is a result of movement through waterways at the retreating edge of glaciers (Dadswell 1974). Therefore, its presence may be related to that of *M. relicta* for zoogeographic, rather than ecological, reasons.

Fig. 1. Particulate phosphorus slopes for lake groups in the Huntsville–Bancroft region of Ontario and for Lake Ontario, 1992. Slopes are shown averaged over seasons and depths, and for the epilimnion (EP) and metalimnion (MET) for each season. N, lakes without *M. relicta* and planktivorous fish; F, lakes with planktivorous fish; M/F, lakes with *M. relicta* and planktivorous fish; LO, Lake Ontario. Error bars represent 95% confidence limits.

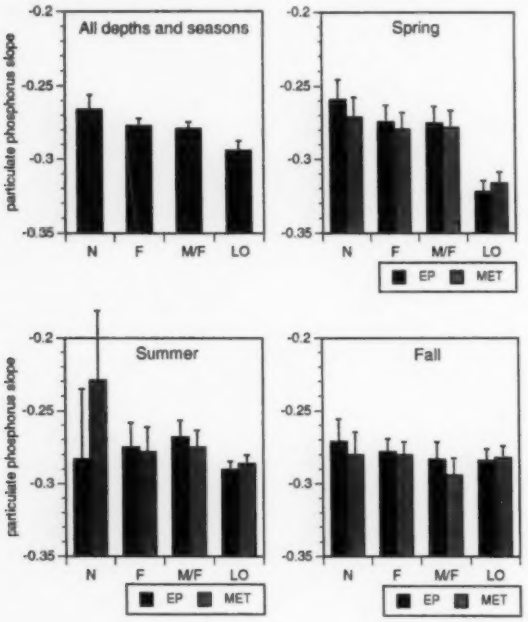
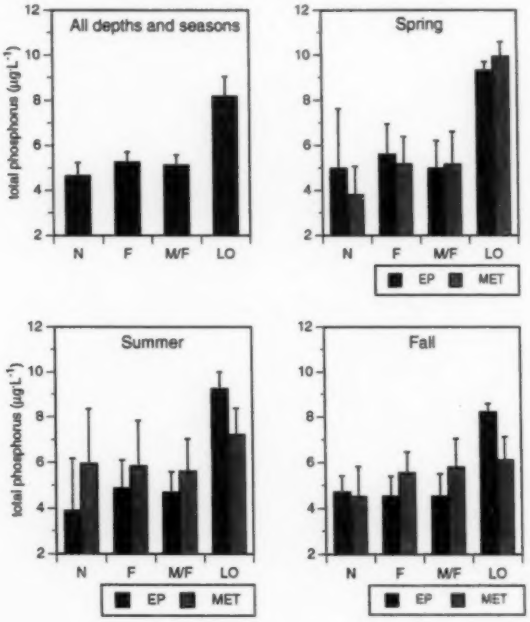


Fig. 2. Total phosphorus (TP) concentrations for lake groups in the Huntsville–Bancroft region of Ontario and for Lake Ontario, 1992. TP concentrations are shown averaged over seasons and depths, and for the epilimnion (EP) and metalimnion (MET) for each season. N, lakes without *M. relicta* and planktivorous fish; F, lakes with planktivorous fish; M/F, lakes with *M. relicta* and planktivorous fish; LO, Lake Ontario. Error bars represent 95% confidence limits.



Epischura lacustris occurred in relatively low numbers and would have been poorly quantified by our counting protocol.

Results

Size distribution of particulate phosphorus

PP slopes, which characterized the size distribution of PP, were different for lakes with and without planktivores in the Huntsville–Bancroft region ($P = 0.02$, RM-ANOVA) when averaged over depths and seasons (Fig. 1). There was, on average, a greater proportion of large plankton in lakes without *M. relicta* and planktivorous fish than in lakes with planktivores. Lakes containing planktivorous fish, but not *M. relicta*, had intermediate PP slopes, but the mean was not significantly different ($P < 0.05$) from that for lakes with *M. relicta* and planktivorous fish.

PP slopes indicated that the proportion of large particles was generally higher in the epilimnion (Fig. 1). However, in the summer, lakes without *M. relicta* and planktivorous fish had significantly larger plankton in the metalimnion than in the epilimnion ($P = 0.003$), although a large variance was associated with these lakes (Fig. 1).

Smaller particle-size distributions were found in Lake Ontario than in any of the lakes from the Huntsville–Bancroft region when distributions were averaged over depths and seasons (Fig. 1). This difference was strongest in the spring for both the epilimnion and metalimnion and weakest in the fall, diminishing through the summer (Fig. 1). Within Lake Ontario, the size distribution for the epilimnion was generally smaller than in the metalimnion (Fig. 1).

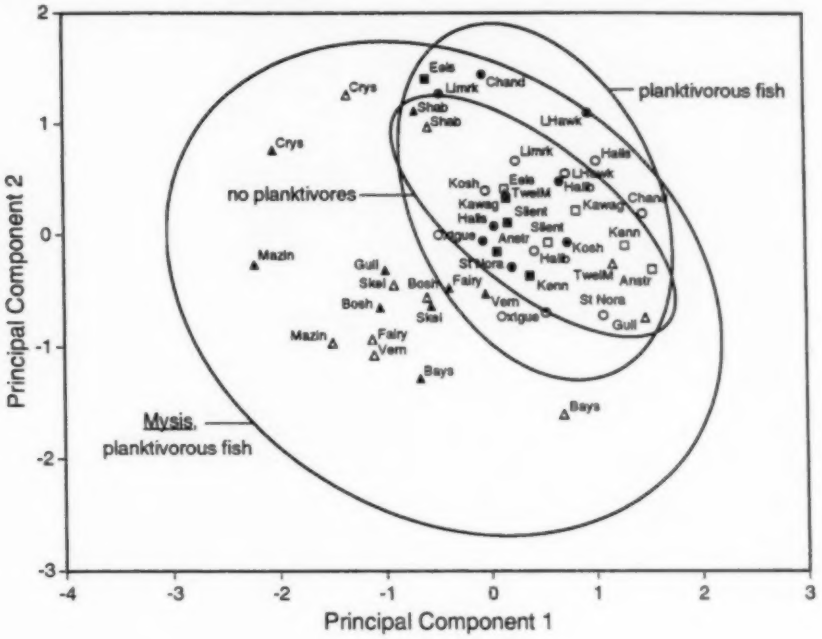
Total phosphorus

When averaged over depths and seasons, there were no differences in TP concentration between lakes in the Huntsville–Bancroft region with and without *M. relicta* and planktivorous fish ($P = 0.69$, RM-ANOVA) (Fig. 2). The range of TP concentration within these inland lakes was relatively small, only about 2 to 10 µg $P \cdot L^{-1}$. TP concentrations for Lake Ontario were at the high end of this range, but nevertheless within the range. Depth and season had no strong overall effect on TP concentrations. TP concentrations were not correlated with the size distribution of plankton.

Zooplankton species composition and size distribution

To reduce the number of variables in the principal components and discriminant functions analyses, the biomasses of similar species were combined. The following species, or groups of species, were included in the analyses: bosminids, *Chydorus sphaericus*, small *Daphnia* spp., large *Daphnia* spp., *Diaphanosoma brachyurum*, *Holopedium gibberum*, *Sida crystallina*, calanoid copepods, and cyclopoid copepods. Bosminids included *Bosmina longirostris*, *Eubosmina tubicen*, and

Fig. 3. Principal components ordination of zooplankton biomass for lakes in the Huntsville–Bancroft region of Ontario. Open and solid symbols represent summer and fall, respectively. Squares, lakes without *M. relicta* and planktivorous fish; circles, lakes with planktivorous fish; triangles, lakes with *M. relicta* and planktivorous fish. Ellipses represent 95% confidence regions for the data values for each lake group.



Eubosmina longispina. Small *Daphnia* included *Daphnia longiremis* and *Daphnia retrocurva*. Large *Daphnia* included *Daphnia dubia*, *Daphnia galeata mendotae*, and *Daphnia pulex*. Calanoid copepods included copepodites, *Leptodiaptomus minutus*, and *Skistodiaptomus oregonensis*. Cyclopoid copepods included copepodites, *Diacyclops thomasi*, *Cyclops scutifer*, and *Mesocyclops edax*. *Daphnia longiremis* and *Daphnia retrocurva* were classified as small because they range in size from 0.8 to 1.2 mm and from 1.2 to 1.8 mm, respectively (Balcer et al. 1984). *Daphnia dubia*, *Daphnia galeata mendotae*, and *Daphnia pulex* were classified as large because they range in size from 1.5 to 2 mm, from 1.3 to 3.0 mm, and from 1.3 to 2.2 mm, respectively (Brooks 1957; Balcer et al. 1984).

The biomass of zooplankters was transformed using $\log(X + 0.1)$, where X is the biomass variable. A plot of variance against mean for each of the transformed variables within the lake groups suggested that the variances no longer varied directly with the means. Transformations were not required for zooplankton length and the proportion of zooplankton biomass contributed by zooplankton longer than 1 mm because the variances were not correlated with the means.

In most of the lakes we sampled, calanoid copepodites dominated the zooplankton communities in terms of biomass, followed by *Daphnia galeata mendotae*, cyclopoid copepodites, bosminids, and *Diacyclops thomasi*. In lakes without planktivores, *H. gibberum* generally comprised a larger proportion of the biomass than in lakes with planktivores.

Principal components were derived from the zooplankton species covariance matrix and used to determine if the lakes fell into groups on the basis of the presence or absence of planktivores (Fig. 3). The first two principal components expressed 49% of the total variance in the data (Table 2). Small *Daphnia* spp. and *H. gibberum* had the highest positive loadings on the first principal component, whereas *Diaphanosoma brachyurum* showed the strongest negative loading. *Sida crystallina* had the highest positive loading on the second principal component. Lakes with *M. relicta* and planktivorous fish were mostly in the lower left of the ordination, but the presence of *M. relicta* and planktivorous fish did not appear to be the major factor determining the zooplankton species composition of these communities.

Table 2. Correlations of zooplankton biomass with principal components 1 and 2.

Zooplankton	PC1	PC2
Bosminids	0.295	−0.266
Calanoid copepods	0.082	0.196
Chydorus sphaericus	0.004	0.076
Cyclopoid copepods	0.131	0.127
Daphnia spp. (small)	0.466	0.221
Daphnia spp. (large)	0.297	0.345
Diaphanosoma brachyurum	−0.281	0.151
Holopedium gibberum	0.584	−0.249
Sida crystallina	0.070	0.403
% variance	29.6	19.2

Fig. 4. Discriminant functions analysis of zooplankton biomass for lakes in the Huntsville–Bancroft region of Ontario. Data for mean *Daphnia* length, total zooplankton biomass, and proportion of zooplankton biomass longer than 1 mm are included in the analysis. Open and solid symbols represent summer and fall, respectively. Squares, lakes without *M. relicta* and planktivorous fish; circles, lakes with planktivorous fish; triangles, lakes with *M. relicta* and planktivorous fish. Ellipses represent 95% confidence regions for the data values for each lake group.

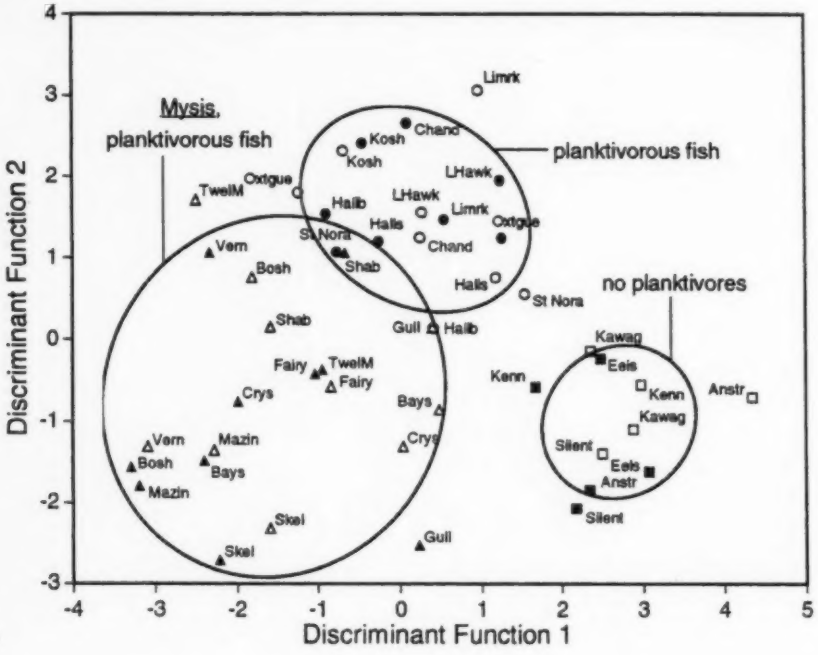


Table 3. For lakes in the Huntsville–Bancroft region of Ontario, discriminant function loadings (DF) of biomass of various individual and groups of zooplankters.

Zooplankton	*F*	*P*	DF1	DF2
(Biomass of cladocerans and copepods over 1 mm)/(total biomass)	23.584	0.000	0.619	0.158
Daphnia spp. (small)	19.508	0.000	0.253	0.723
(Biomass of cladocerans over 1 mm)/(total biomass)	14.530	0.000	0.479	0.168
Daphnia spp. (large)	9.817	0.000	0.363	0.256
Mean length of *Daphnia* spp.	7.753	0.001	0.354	–0.093
Total biomass of zooplankton	6.737	0.003	0.313	0.174
Holopedium gibberum	5.051	0.011	0.290	–0.037
Cyclopoid copepods	3.718	0.032	0.057	0.343
Calanoid copepods	3.142	0.053	0.213	–0.121
Sida crystallina	1.826	0.173	0.145	0.137
Bosminids	1.649	0.204	–0.079	0.206
Diaphanosoma brachyurum	1.458	0.244	–0.151	–0.056
Chydorus sphaericus	0.897	0.415	–0.083	0.127

Note: Derived variables, including mean *Daphnia* length, total zooplankton biomass, and proportion of zooplankton biomass over 1 mm, are also included. *F* statistics and probability (*P*) values are based on one-way ANOVAs.

Discriminant functions analysis was then used to determine if the three predetermined lake groups, defined on the basis of the presence or absence of planktivores, could be distinguished from one another by the species composition of zooplankton. The predetermined groups included lakes without *M. relicta* and planktivorous fish, lakes with planktivorous fish but without *M. relicta*, and lakes with *M. relicta* and planktivorous fish. Discriminant functions analysis did sort the lakes into overlapping groups with and without planktivores (data not shown). However, the best separation occurred when derived variables that more directly assessed zooplankton biomass and size were added to the analysis (Fig. 4).

The variables most successful at separating the lakes were the proportion of zooplankton biomass contributed by cladocerans and copepods longer than 1 mm and the proportion of zooplankton biomass contributed by cladocerans longer than 1 mm, as indicated by their high *F* values and positive loadings on the first discriminant function (Table 3). The biomass of small *Daphnia* spp. also had a high *F* value and was associated with the second discriminant function, which separated lakes with planktivorous fish but without *M. relicta* from the other lakes.

When projected onto the discriminant functions analysis, stations in Lake Ontario were sorted into the same group as those lakes from the Huntsville–Bancroft region containing both *M. relicta* and planktivorous fish. However, within this

Fig. 5. Separation between lakes with and without planktivorous fish in the Huntsville–Bancroft region of Ontario using discriminant functions analysis. Data for mean *Daphnia* length, total zooplankton biomass, and proportion of zooplankton biomass longer than 1 mm are included in the analysis.

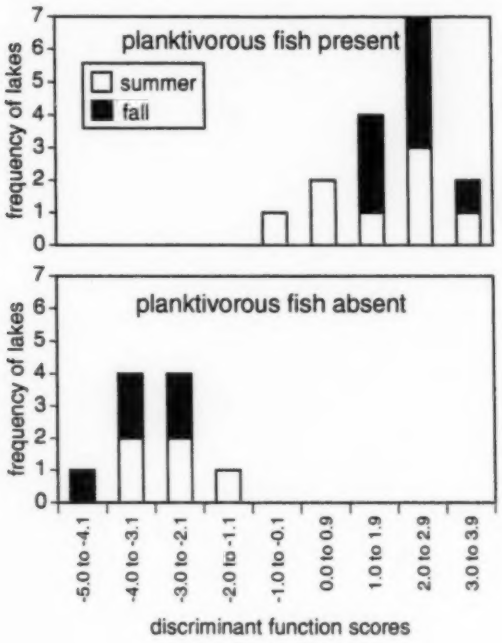
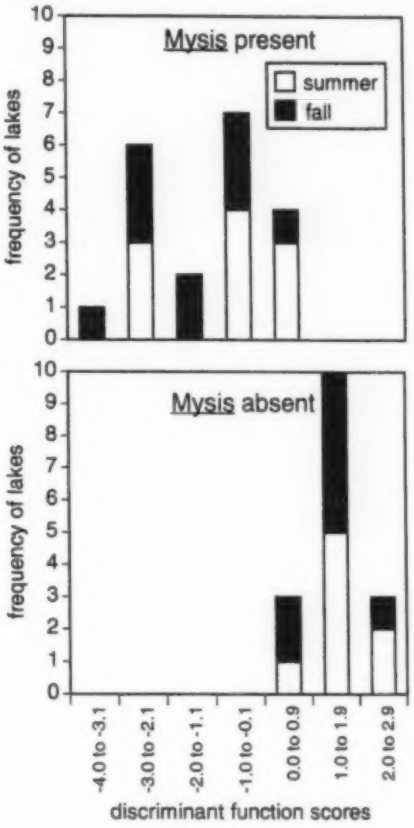


Table 4. For lakes with and without planktivorous fish in the Huntsville–Bancroft region of Ontario, discriminant function loadings (DF) of biomass of various individual and groups of zooplankters.

Zooplankton	*F*	*P*	DF
Daphnia spp. (small)	18.337	0.000	0.345
Mean length of *Daphnia* spp.	11.045	0.003	–0.268
(Biomass of cladocerans and copepods over 1 mm)/(total biomass)	7.094	0.014	–0.215
Bosminids	5.398	0.029	0.187
Cyclopoid copepods	5.336	0.030	0.186
Holopedium gibberum	4.495	0.045	–0.171
Calanoid copepods	3.565	0.071	–0.152
(Biomass of cladocerans over 1 mm)/(total biomass)	3.530	0.072	–0.151
Chydorus sphaericus	1.519	0.230	0.099
Total biomass of zooplankton	0.975	0.333	–0.080
Daphnia spp. (large)	0.953	0.339	–0.079
Diaphanosoma brachyurum	0.374	0.547	0.049
Sida crystallina	0.000	0.997	–0.000

Note: Derived variables, including mean *Daphnia* length, total zooplankton biomass, and proportion of zooplankton biomass over 1 mm, are also included. *F* statistics and probability (*P*) values are based on one-way ANOVAs.

Fig. 6. Separation between lakes with and without *M. relicta* for lakes in the Huntsville–Bancroft region of Ontario using discriminant functions analysis. Data for mean *Daphnia* length, total zooplankton biomass, and proportion of zooplankton biomass longer than 1 mm are included in the analysis.



group, Lake Ontario stations were placed apart from the other lakes because the proportion of zooplankton biomass contributed by zooplankters longer than 1 mm was lower than that for any of the other lakes. Lake Ontario also had a higher biomass of small *Daphnia* spp. and cyclopoid copepods than the other lakes, particularly in the fall.

To isolate the effects of planktivorous fish on the size distribution of zooplankton, lakes without *M. relicta* but with and without planktivorous fish were examined separately. Discriminant functions analysis successfully separated lakes with planktivorous fish from lakes without planktivorous fish (Fig. 5) mainly on the basis of the biomass of small *Daphnia* spp. and the mean length of *Daphnia* spp. (Table 4). Lakes with planktivorous fish had a higher biomass of small *Daphnia* spp. and a smaller mean length for *Daphnia* spp. than lakes without planktivorous fish. Next, we isolated the effects of *M. relicta* by examining lakes with planktivorous fish but with and without *M. relicta*. Discriminant functions analysis separated lakes with *M. relicta* from lakes without *M. relicta* with some overlap (Fig. 6). The most useful variables were the biomass of small *Daphnia* spp., the proportion of biomass

Table 5. For lakes with and without *Mysis relicta* in the Huntsville–Bancroft region of Ontario, discriminant function loadings (DF) of biomass of various individual and groups of zooplankters.

Zooplankton	*F*	*P*	DF
Daphnia spp. (small)	36.199	0.000	0.725
(Biomass of cladocerans and copepods over 1 mm)/(total biomass)	17.131	0.000	0.499
(Biomass of cladocerans over 1 mm)/(total biomass)	11.930	0.001	0.416
Daphnia spp. (large)	11.081	0.002	0.401
Total biomass of zooplankton	6.916	0.013	0.317
Cyclopoid copepods	6.307	0.017	0.303
Sida crystallina	3.484	0.071	0.225
Holopedium gibberum	1.457	0.236	0.146
Mean length of *Daphnia* spp.	1.352	0.253	0.140
Diaphanosoma brachyurum	1.174	0.286	−0.131
Bosminids	0.994	0.236	0.120
Chydorus sphaericus	0.150	0.701	0.047
Calanoid copepods	0.071	0.792	0.032

Note: Derived variables, including mean *Daphnia* length, total zooplankton biomass, and proportion of zooplankton biomass over 1 mm, are also included. *F* statistics and probability (*P*) values are based on one-way ANOVAs.

contributed by zooplankton longer than 1 mm, and the biomass of large *Daphnia* spp. (Table 5). Lakes with *M. relicta* had lower biomasses of both small and large *Daphnia* spp. and smaller proportions of zooplankton biomass longer than 1 mm than lakes without *M. relicta*. Although *M. relicta* and fish had similar effects on larger *Daphnia* spp., they had the opposite effect on smaller *Daphnia* spp. (i.e., there were higher biomasses of small *Daphnia* spp. when both *M. relicta* and fish were present).

Discussion

Effect of planktivores on plankton community structure

The size distribution of particulate phosphorus indicates a greater proportion of large particles in lakes without planktivores. There was no discernible difference in particle-size distribution between lakes with planktivorous fish and lakes with both *M. relicta* and planktivorous fish. The differences between lakes with and without *M. relicta* that were evident from examination of the zooplankton were largely in the relative abundance of large and small Cladocera, especially *Daphnia* spp. However, because the largest size-class in the particle-size technique is >400 µm, perhaps it is not sensitive to these more subtle effects of *M. relicta*. Also, because a relatively low volume of water was filtered in the particle-size technique, relative to the net hauls, the density and size structure of the mesoplankton may have been poorly represented using this technique (Prepas and Vickery 1984).

Lakes with planktivorous fish had a larger biomass of small *Daphnia* spp. and a smaller mean length of *Daphnia* spp. than lakes without planktivorous fish. This agrees with earlier studies in which size-selective planktivorous fish have been shown to select large *Daphnia* spp. (Brooks and Dodson 1965; Kerr 1971; Langeland et al. 1991; Rudstam et al. 1993). In so doing, planktivorous fish may release small *Daphnia* spp. from competition. Lakes with *M. relicta* had a lower biomass of both small and large *Daphnia* spp., a smaller proportion of zooplankton biomass longer than 1 mm, a lower biomass of cyclopoid copepods, and a lower overall biomass of zooplankton than lakes without *M. relicta*. Similar responses to *M. relicta* have been observed in previous studies. For instance, Langeland et al. (1991) found that in White Partridge Lake, Ontario, which has a natural population of *M. relicta*, and in Lake Selbusjoen, Norway, which has an introduced population of *M. relicta*, the biomass and size distribution of cladocerans were smaller than in Lake Opeongo and Lake Louisa, Ontario, in which *M. relicta* is absent. Nero and Sprules (1986) compared two lakes in central Ontario containing endemic populations of *M. relicta* with two lakes without *M. relicta*. In the lakes with *M. relicta*, there was a lower abundance of hypolimnetic zooplankters, including the cladocerans *Daphnia longiremis* and *Eubosmina longispina*, and the copepods *Cyclops scutifer* and *Onychodiaptomus sanguineus*. In lakes with *M. relicta*, there was a higher abundance of the cladoceran *Diaphanosoma* spp in the metalimnion, which may have been brought about by a decrease in the abundance of its competitor, *Daphnia* spp. Our results suggest that *M. relicta* and planktivorous fish not only consume large cladocerans but that *M. relicta* also consumes smaller cladocerans than those consumed by planktivorous fish. This may be due to the size distribution of *M. relicta* present in the lakes. In laboratory studies, Cooper and Goldman (1980) demonstrated that larger *M. relicta* showed a preference for larger prey such as *Epischura* spp. and *Daphnia* spp., whereas smaller *M. relicta* selected smaller prey such as *Bosmina* spp.

The presence or absence of *M. relicta* and planktivorous fish did not appear to be the major factors determining the species composition of zooplankton communities in these lakes; the presence or absence of planktivores did not correspond strongly with the principal components of variation in species composition. A separation of lakes by planktivores occurred only when discriminant functions analysis was used, which seeks to maximize differences between predetermined groups, and then succeeded best when derived variables reflecting zooplankton size were included. One of the most important factors in determining prey selection by particulate feeders is visibility, which is influenced by the contrast between the prey item and its background, the conspicuousness of the prey, and the visual acuity of the planktivore (Lazarro 1987); however, body shape (Kerfoot et al. 1980), eye size (Zaret 1972), body transparency (O'Brien et al. 1979), and swimming motion have also been shown to be important. In general, however, the most visible zooplankters are consumed by selective planktivores (Zaret 1980). We conclude that factors other than size-selective planktivory may be the most important determinants of species composition, and (or) that the presence or absence of these planktivores is a poor indication of the intensity of size-selective planktivory.

Because of the patchy horizontal and vertical distribution of zooplankton, unrepresentative zooplankton samples could lead to a misinterpretation of species composition and size structure. This was of particular concern in this study because only a single sample was collected on each of two visits to each lake. However, a simultaneous study of spatial distribution

using zooplankton samples collected along a transect in 12 of the 23 lakes that were sampled in the Huntsville– Bancroft region showed that the between-lake variation in zooplankton size structure was greater than the within-lake variation (M.N. Futter, Trent University, personal communication). Guy et al. (1994) also found that the between-lake variability of the size distribution of PP for 10 lakes was stronger than the temporal variability.

The PP slopes obtained in this study agree with those observed in previous studies in which slope values ranged from approximately –0.2 for lakes and enclosures with abundant large plankton and low planktivory to –0.3 for lakes and enclosures with abundant small plankton and high planktivory (Mazumder et al. 1988, 1990; Drenner and Smith 1991). When season and depth data were averaged for the inland lakes, slopes ranged from –0.26 to –0.28 (mean = –0.27) for lakes without planktivores, from –0.27 to –0.29 (mean = –0.28) for lakes with planktivorous fish, and from –0.27 to –0.29 (mean = –0.28) for lakes with *M. relicta* and planktivorous fish. However, PP slopes from these lakes generally fell within a limited range, and most slopes were steep, indicative of a high proportion of small particles. Guy et al. (1994), working on 10 lakes in the same area and including three of the 23 lakes we studied, found a similar limited range of values when seasonal means were considered. The present study extended the range of observed slopes to include some in the range of approximately –0.2, the extreme previously observed in fishless enclosures. These low slope values were not common, and most slopes <–0.26 occurred in lakes without planktivores, particularly in the summer metalimnion.

Stations from Lake Ontario were most similar to lakes in the Huntsville–Bancroft region containing *M. relicta* and planktivorous fish, both in size distribution and in zooplankton composition. However, Lake Ontario was extreme in both respects. The proportion of biomass contributed by cladocerans and copepods longer than 1 mm was much lower in Lake Ontario than for the other lakes and the biomass of small *Daphnia* spp. and cyclopoid copepods was higher than for the other lakes. These differences were more pronounced in the fall. Other Lake Ontario studies have also reported a predominance of cyclopoid copepods, small bosminids, small *Daphnia* spp., and a low overall zooplankton biomass (Watson and Carpenter 1974; Taylor et al. 1987; Johannsson and O'Gorman 1991; Mazumder et al. 1992).

Because of the wide-scale nature of this study, we are dealing with global features of the lake groups and therefore certain assumptions have been made. For instance, the species composition of the fish communities was primarily based on previous surveys that may not accurately represent the current community structure. The patterns we uncovered may represent direct effects of *M. relicta* and planktivorous fish. However, factors related to the presence or absence of *M. relicta* and planktivorous fish in the study lakes, such as lake morphometry and lake chemistry, may also be influencing the zooplankton community structure independent of planktivory.

Size distribution of PP versus zooplankton counts

The size-selective effect of planktivores was detected using both the size distribution of PP, characterized by PP slopes, and the more traditional method of counting and measuring zooplankters. However, lakes with *M. relicta* and planktivorous fish were not separated from lakes with planktivorous fish using the size distribution of PP but they were separated from lakes with planktivorous fish using the count method. The slope parameter, obtained by the filter fractionation of PP, and the proportion of biomass contributed by zooplankton longer than 1 mm, obtained by using the direct count method, are both continuous variables with the potential to estimate the intensity of predation by planktivores. Therefore, as indicators of food web structure, they also may have the potential to predict bioaccumulation of persistent organochlorines.

The PP method has the advantage of being less time consuming and tedious than the preservation, counting, and measurement of zooplankton. Additional data, however, might enhance the effectiveness of the PP slope parameter for comparing communities and predicting organochlorine bioaccumulation. For instance, sonar techniques could be used to estimate the abundance of planktivorous fish and therefore the intensity of predation. *Daphnia* mortality rates also could be obtained to assess the intensity of planktivory. A direct relationship between the PP slope parameter and these additional variables could help to justify the use of the slope parameter as an indicator of the intensity of predation by planktivores. Furthermore, the size distribution of PP could be extended to include size-classes larger than 400 μm. Plankton samples obtained from net hauls could be filtered through screens with different mesh sizes and analysed for phosphorus. If, however, a more detailed analysis of the effect of planktivory is required, the direct count method is warranted. It, no doubt, can provide additional information that is lost by using the size distribution of PP, such as life-history data and species composition.

Effect of planktivores on vertical structure

By separately sampling the epilimnion and metalimnion, information was obtained that would otherwise have gone undetected by taking integrated samples. Lakes without *M. relicta* and planktivorous fish had a significantly higher proportion of large particles in the metalimnion than in the epilimnion during the summer. Higher TP values were also observed in the metalimnion than the epilimnion during the summer for lakes without planktivores, which suggests that the higher proportion of large particles was due to an abundance of large plankters rather than a reduction in the abundance of small plankters. Lake Ontario also generally had a higher proportion of large particles in the metalimnion than in the epilimnion. However, in the summer and fall, TP was lower in the metalimnion than in the epilimnion, which suggests that the higher proportion of large particles in the metalimnion resulted from a reduction in the abundance of small particles, rather than an increase in the abundance of large particles.

The higher proportion of large particles in the metalimnion of the smaller lakes may indicate metalimnetic maxima in primary production and (or) algal biomass. Certain planktonic algae, such as dinoflagellates and chrysophytes, are motile and show phototaxis, while some Cyanobacteria can regulate their buoyancy. Therefore, they tend to accumulate at certain depths where turbulence is weak and light penetration and nutrients levels are sufficient to allow growth (Pick and Lean 1984). Large zooplankton may inhabit this region to take advantage of the productivity and may, therefore, have contributed to the higher proportion of large particles. Large inedible

phytoplankton, such as the filamentous blue-green alga *Oscillatoria rubescens*, may also have contributed to the higher proportion of large particles. Because of their elongate shape, they could become trapped on the 200- and 400-μm screens. However, phytoplankton were not noticeably abundant in the zooplankton samples taken with a 200-μm net, which suggests that the higher proportion of large particles in metalimnetic samples was due mainly to the presence of zooplankton.

The low abundance of plankton in the epilimnion of lakes without planktivores may actually have favoured the growth of phytoplankton in the metalimnion. Mazumder et al. (1988, 1990) reported that water clarity improved in fishless enclosures. Grazing by large zooplankton is associated with a decrease in the abundance of nanoplankton; in the absence of these smaller particles, light attenuation may be reduced. As a result of this improved water clarity, light penetration may increase in the water column, with associated increases in epilimnetic depth and metalimnetic temperature (Mazumder and Taylor 1994).

The higher proportion of large particles in the metalimnion may also have been related to the diel migration patterns of zooplankton. Larger zooplankton have been shown to migrate deeper in the water column during the day than smaller zooplankton (Hutchinson 1967) to seek refuge from visual planktivores in regions of lower light intensity (Zaret and Suffern 1976; Wright et al. 1980). However, the euphotic zone did extend into the metalimnion in most of the lakes studied.

These data indicate differences among lakes in plankton communities that are related to the presence of *M. relicta* and planktivorous fish and link the results obtained by Rasmussen et al. (1990) for lake trout with those of Taylor et al. (1991) for net plankton. That is, lakes with planktivores have more contaminated lake trout (Rasmussen et al. 1990), lower zooplankton biomass (this study), and more contaminated zooplankton (Taylor et al. 1991). The differences among these lakes in contaminant bioaccumulation by lake trout could result from differences in food webs leading from zooplankton to trout, and from differences at lower trophic levels up to and including zooplankton.

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References

Balcer, M.D., Korda, N.L., and Dodson, S.I. 1984. Zooplankton of the Great Lakes: a guide to the identification and ecology of the common crustacean species. The University of Wisconsin Press, Madison, Wis.

Brooks, J.L. 1957. The systematics of North American *Daphnia*. Mem. Conn. Acad. Arts Sci. **13**: 1–180

Brooks, J.L. 1968. The effects of prey size selection by lake planktivores. Syst. Zool. **17**: 272–291.

Brooks, J.L., and Dodson, S.I. 1965. Predation, body size and composition of plankton. Science (Washington, D.C.), **150**: 28–35.

Cooper, S.D., and Goldman, C.R. 1980. Opossum shrimp (*Mysis relicta*) predation on zooplankton. Can. J. Fish. Aquat. Sci. **37**: 909–919.

Crowder, M.J., and Hand, D.J. 1990. Analysis of repeated measures. Chapman and Hall, London.

Culver, D.A., Boucherle, M.M., Bean, D.J., and Fletcher, J.W. 1985. Biomass of freshwater crustacean zooplankton from length–weight regressions. Can. J. Fish. Aquat. Sci. **42**: 1380–1390.

Dadswell, M.J. 1974. Distribution ecology and postglacial dispersal of certain crustaceans and fishes in eastern North America. *In* Publications in zoology. No. 11. National Museum of Natural Sciences, Ottawa, Ont.

Drenner, R.W., and Smith, J.D. 1991. Biomass dependent effects of mosquitofish on zooplankton, chlorophyll and the size distribution of particulate phosphorus. Verh. Int. Ver. Angew. Limnol. **24**: 2382–2386.

Evans, D.O., and Loftus, D.H. 1987. Colonization of inland lakes in the Great Lakes region by rainbow smelt *Osmerus mordax*: their freshwater niche and effects on indigenous fishes. Can. J. Fish. Aquat. Sci. **44**: 249–266.

Gardner, M.B. 1981. Mechanisms of size selectivity by planktivorous fish: a test of hypotheses. Ecology, **62**: 571–578.

Guy, M., Taylor, W.D., and Carter, J.C.H. 1994. Decline in total phosphorus in the surface waters of lakes during summer stratification, and its relationship to size distribution of particles and sedimentation. Can. J. Fish. Aquat. Sci. **51**: 1330–1337.

Hutchinson, G.E. 1967. A treatise on limnology. Vol. 2. Introduction to lake biology and the limnoplankton. John Wiley and Sons, New York.

Johannsson, O.E., and O'Gorman, R. 1991. Roles of predation, food, and temperature in structuring the epilimnetic zooplankton populations in Lake Ontario, 1981–1986. Trans. Am. Fish. Soc. **120**: 193–208.

Kerfoot, W.C., Kellogg, D.L., Jr., and Strickler, J.R. 1980. Visual observations of live zooplankters: evasion, escape, and chemical defenses. *In* Evolution and ecology of zooplankton communities. Spec. Symp. Vol. 3. *Edited by* W.C. Kerfoot. American Society of Limnology and Oceanography, University Press of New England, Hanover, N.H. pp. 10–27.

Kerr, S.R. 1971. Prediction of fish growth efficiency in nature. J. Fish. Res. Board Can. **28**: 809–814.

Kerr, S.R., and Martin, N.V. 1970. Trophic-dynamics of lake trout production systems. *In* Marine food chains. *Edited by* J. H. Steele. Oliver and Boyd, Edinburgh, U.K. pp. 365–376.

Langeland, A., Carl, L.M., Hicks, F.J., and Monroe, B. 1991. Impact of predation by *Mysis relicta* and fish on zooplankton in four oligotrophic, north temperate lakes. Am. Fish. Soc. Symp. **9**: 88–97.

Lazarro, X. 1987. A review of planktivorous fishes: their evolution, feeding behaviours, selectivites, and impacts. Hydrobiologia, **146**: 97–167.

Lean, D.R.S., Fricker, H.-J., Charlton, M.N., Cuhel, R.L., and Pick, F.R. 1987. The Lake Ontario life support system. Can. J. Fish. Aquat. Sci. **44**: 2230–2240.

Mazumder, A., and Taylor, W.D. 1994. Thermal structure of lakes varying in size and water clarity. Limnol. Oceanogr. **39**: 968–976.

Mazumder, A., McQueen, D.J., Taylor, W.D., and Lean, D.R.S. 1988. Effects of fertilization and planktivorous fish (yellow perch) predation on size distribution of particulate phosphorus and assimilated phosphate: large enclosure experiments. Limnol. Oceanogr. **33**: 421–430.

Mazumder, A., Taylor, W.D., McQueen, D.J., Lean, D.R.S., and Lafontaine, N.R. 1990. A comparison of lakes and lake enclosures with contrasting abundances of planktivorous fish. J. Plankton Res. **12**: 109–124.

Mazumder, A., Lean, D.R.S., and Taylor, W.D. 1992. Dominance of small filter feeding zooplankton in the Lake Ontario foodweb. J. Great Lakes Res. **18**: 456–466.

McCauley, E. 1984. The estimation of the abundance and biomass of zooplankton in samples. *In* A manual on methods for the assessment of secondary productivity in fresh waters. 2nd ed. *Edited by* J.A. Downing and F.H. Rigler. Blackwell Scientific Publications, Oxford. pp. 228–265.

Menzel, D.W., and Corwin, N. 1965. The measurement of total P in seawater based on the liberation of organically bound fraction by persulfate oxidation. Limnol. Oceanogr. **10**: 280–282.

Nero, R.W., and Sprules, W.G. 1986. Zooplankton species abundance and biomass in relation to occurrence of *Mysis relicta* (Malacostraca: Mysidacea). Can. J. Fish. Aquat. Sci. **43**: 420–434.

O'Brien, W.J., Kettle, D., and Riessen, H. 1979. Helmets and invisible armor: structures reducing predation from tactile and visual planktivores. Ecology, **60**: 287–294.

Pick, F.R., and Lean, D.R.S. 1984. Diurnal movements of metalimnetic phytoplankton. J. Phycol. **20**: 430–436.

Prepas, E.E., and Vickery, J. 1984. Contribution of particulate phosphorus (>250 μm) to the total phosphorus pool in lake water. Can. J. Fish. Aquat. Sci. **41**: 351–363.

Rasmussen, J.B., Rowan, D.J., Lean, D.R.S., and Carey, J.H. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Can. J. Fish. Aquat. Sci. **47**: 2030–2038.

Reckahn, J.A. 1970. Ecology of young lake whitefish (*Coregonus clupeaformis*) in South Bay, Manitoulin Island, Lake Huron. *In* Biology of coregonid fishes. *Edited by* C.C. Lindsey and C.S. Woods. University of Manitoba Press, Winnipeg, Man. pp. 437–460.

Rudstam, L.G., Lathrop, R.C., and Carpenter, S.R. 1993. The rise and fall of a dominant planktivore: direct and indirect effects on zooplankton. Ecology, **74**: 303–319.

Sprules, W.G. 1984. Towards an optimal classification of zooplankton for lake ecosystem studies. Verh. Int. Ver. Theor. Angew. Limnol. **22**: 320–325.

Steel, R.G.D., and Torrie, J.H. 1960. Principles and procedures of statistics with special reference to the biological sciences. McGraw-Hill Book Company, Inc., Toronto, Ont.

Strickland, J.D., and Parsons, T.R. 1972. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. No. 167.

Strus, R.H., and Hurley, D.A. 1992. Interactions between alewife (*Alosa pseudoharengus*), their food, and phytoplankton biomass in the Bay of Quinte, Lake Ontario. J. Great Lakes Res. **18**: 709–723.

Taylor, W.D., Fricker, H.-J., and Lean, D.R.S. 1987. Zooplankton seasonal succession in Lake Ontario at northshore, midlake, and southshore stations in 1982, and a comparison with 1970. Can. J. Fish. Aquat. Sci. **44**: 2178–2184.

Taylor, W.D., Carey, J.H., Lean, D.R.S., and McQueen, D.J. 1991. Organochlorine concentrations in the plankton of lakes in southern Ontario and their relationship to plankton biomass. Can. J. Fish. Aquat. Sci. **48**: 1960–1966.

Trippel, E.A., and Beamish, F.W.H. 1989. Lake trout (*Salvelinus namaycush*) growth potential predicted from cisco (*Coregonus artedii*) population structure and conductivity. Can. J. Fish. Aquat. Sci. **46**: 1531–1538.

Vanni, M.J., Luecke, C., Kitchell, J.F., Allen, Y., Temte, J., and Magnuson, J.J. 1990. Effects on lower trophic levels of massive fish mortality. Nature (London), **344**: 333–335.

Watson, N.H.F., and Carpenter, G.F. 1974. Seasonal abundance of crustacean zooplankton and net plankton biomass of lakes Huron, Erie, and Ontario. J. Fish. Res. Board Can. **31**: 309–317.

Werner, E.E., and Hall, D.J. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). Ecology, **55**: 1042–1052.

Wilkinson, L. 1992. SYSTAT: statistics, version 5.2 edition. SYSTAT, Inc., Evanston, Ill.

Winer, B.S. 1971. Statistical principles in experimental design. 2nd ed. McGraw-Hill, Inc., New York.

Wright, D., O'Brien, W.J., and Vinyard, G.L. 1980. Adaptive value of vertical migration: a simulation model argument for the predation hypothesis. *In* Evolution and ecology of zooplankton communities. *Edited by* W.C. Kerfoot. University Press of New England, Hanover, N.H. pp. 138–147.

Yan, N.D., and Mackie, G.L. 1987. Improved estimation of the dry weight of *Holopedium gibberum* (Crustacea, Cladocera) using clutch size, a body fat index, and lake water total phosphorus concentration. Can. J. Fish. Aquat. Sci. **44**: 382–389.

Zaret, T.M. 1972. Predators, invisible prey, and the nature of polymorphism in the Cladocera (class Crustacea). Limnol. Oceanogr. **17**: 171–184.

Zaret, T.M. 1980. Predation and freshwater communities. Yale University Press, New Haven, Conn.

Zaret, T.M., and Suffern, J.S. 1976. Vertical migration in zooplankton as a predator avoidance mechanism. Limnol. Oceanogr. **21**: 804–813.

Adipocytes and lipid distribution in the muscle tissue of Atlantic salmon (*Salmo salar*)

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Abstract: Histological studies were conducted to examine the adipocyte and lipid distribution in different parts of muscle tissues and mesenteric tissues of Atlantic salmon (*Salmo salar*). Belly flaps, subcutaneous fat layer, and myosepta were found to be the main sites for adipocytes. The density and morphology of the adipocytes were similar in the mesenteric tissue and the belly flaps and no significant difference in their average adipocyte size was observed. In muscle tissue proper, adipocytes were mainly distributed in myosepta and to a lesser extent in connective tissue (perimysium) surrounding bundles of white muscle fibres. In muscle the connective tissue and associated adipocytes decreased sharply from the belly flap region to the leaner dorsal white muscle. Lipid droplets occurred in the endomysium around dark muscle fibres, but were only occasionally observed around dorsal white muscle fibres. Finely dispersed intracellular lipid droplets were present in dark muscle cells but were not observed in dorsal white muscle cells. Morphological studies on the size distribution of adipocytes through both enzymatic isolation and freeze sectioning, and study of frozen sections stained for lipids, gave similar results of a predominance of small adipocytes (20–40 μm).

Résumé : On a effectué des études histologiques afin d'examiner la répartition des adipocytes et des lipides dans diverses parties des tissus musculaires et des tissus mésentériques du saumon atlantique (*Salmo salar*). On a constaté que les parois abdominales, la couche de graisse souscutanée et les septums des faisceaux étaient les principaux sites des adipocytes. La densité et la morphologie des adipocytes étaient semblables dans le tissu mésentérique et dans les parois abdominales, et on n'observait aucune différence significative dans la taille moyenne des adipocytes. Dans les tissus musculaires proprements dits, les adipocytes eux-mêmes étaient principalement distribués dans les septums des faisceaux et, dans une moindre mesure, dans le tissu conjonctif (perimysium) entourant les faisceaux de fibres des muscles blancs. Dans les muscles, on observait une diminution marquée des tissus conjonctifs et des adipocytes associés entre la région des parois abdominales et celle du muscle blanc dorsal plus maigre. On observait des gouttelettes de lipides dans l'endomysium autour des fibres de muscles foncés, mais très peu de celles-ci étaient aperçues près des fibres des muscles blanc dorsaux. On observait des gouttelettes de lipides intracellulaires finement dispersées dans les cellules des muscles sombres, mais non dans celles des muscles blancs dorsaux. Des études morphologiques de la distribution des tailles des adipocytes, effectuées tant par isolation enzymatique que par examen de lames préparées au microtome et colorées, ont donné des résultats semblables, avec une prédominance de petits adipocytes (20–40 μm).
[Traduit par la Rédaction]

Introduction

Connective tissues are defined as the complex of cells and extracellular materials that provide the support and framework for all of the other tissues of the body. In fish, the body is supported more by muscle fibres than by the skeleton, which is not as substantial as in terrestrial animals. The numerous muscle fibres are held together through connective tissue. In mammalian tissues adipocytes have been studied for characteristics such as morphology, size distribution, and metabolism (DeMartinis and Francendese 1982; Johnson and Greenwood 1983; Sinnett-Smith and Waddington 1992). Lipids in fish have generally been studied with respect to their chemical, biochemical, or biological aspects (Robinson and Mead 1973; Sheridan et al. 1983; Polvi and Ackman 1992). Only a few papers report the histological and morphological characteristics of lipid bodies in fish (Yamada and Nakamura 1964; Yamada 1972; Eastman and DeVries 1982; Fauconneau et al. 1993). Farmed Atlantic salmon of commercial size contain up to 12% lipids in the edible muscle, the chief lipid storage site in salmonids (Ackman 1989). Understanding the distribution of adipocytes within the muscle tissue is essential to further studies on their biological function, their chemical and biochemical aspects, and even their influence on the textural quality of muscle tissue.

Loose connective tissue is widely distributed in the fish body and is characterized by a relative predominance of resident cells. Adipocytes are commonly found in loose connective tissue. Our previous chemical analysis quantitatively investigated the storage of lipids in the connective tissue myosepta, a concentrated site for lipid storage (Zhou et al. 1995). We have now studied the histological distribution of adipocytes and lipids in different areas of the muscle tissues, and compared the size distribution of adipocytes in belly flaps, mesenteric tissue, and white muscle obtained from different

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tissues through both enzymatic tissue digestion and histological sectioning procedures.

Materials and methods

Atlantic salmon, *Salmo salar*, were held in aerated seawater (6–8°C) at the Dalhousie University Aquatron and fed up to sacrifice on a diet of Fundy Choice feed (Corey Mills Ltd., Fredericton, N.B.). Two fish were used for histological studies, one a male (2950 g, 61 cm) and the other a maturing female (3180 g, 65 cm). For the enzymatic isolation of adipocytes, one moderate-sized male (2218 g, 58 cm) and one large male (6084 g, 75 cm) were used. Another well-fed smaller salmon (955 g, 41 cm, female) was also used for the enzymatic isolation of adipocytes, but was not included in the calculation of morphological size distribution. All of the fish were transported in chilled seawater to the Canadian Institute of Fisheries Technology at the Technical University of Nova Scotia. They were then killed by a blow to the head and gutted, and the gut cavities were washed thoroughly with water immediately before dissection. The total lipid content of muscle tissue was determined following the procedures of Bligh and Dyer (1959).

Histological observations

Cubes of 1 cm^3 or less were dissected from different areas of the muscle tissue, adipose fin, and mesenteric tissue. The samples were immediately fixed in 4% formaldehyde and 1% glutaraldehyde in phosphate buffer at pH 7.2 for at least 3 days at 3°C.

Fixed samples were rinsed with running tap water and cut into small pieces (about one-fourth of the original fixed tissue size). The sample blocks were immersed in O.C.T. compound (Tissue-TEK) on a sample block holder, and sectioned in a Harris cryostat (IEC Ltd., Needham Heights, Mass.). The temperature of the cryostat chamber was set at –20°C for white muscle and –30°C for other tissue blocks. Sections were then stained with oil red O as described by Humason (1979). Thick sections had a tendency to float off the slides. In this case, the sections were held in a small basket for the staining process and picked up again by subbed slides (Humason 1979) at the final stage of washing. The sections were mounted in glycerin jelly (Fisher Scientific Company, Fairlawn, N.J.) and observed using a Zeiss photomicroscope.

Isolation of adipocytes

Adipocytes were isolated from belly flaps, mesenteric tissue, and white muscle locations illustrated by Zhou et al. (1995). Belly flap pieces were cut about 1.5 cm in width from a side of the half fillet. White muscle was taken from a dorsal portion starting about 2 cm behind the head and a concentration of myoseptum material was obtained. The dissected tissues were immediately placed in a Petri dish containing Krebs–Ringer phosphate buffer with 1% albumin (bovine, fraction V, Sigma Chemical Co., St. Louis, Mo.). The Krebs–Ringer phosphate buffer contained 120 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 17.2 mM HPO_4^{2-}–$H_2PO_4^{1-}$ (sodium salts) and the pH was adjusted to 7.4. The tissues were cut into small pieces in the Petri dish and added to 4-oz (1 oz = 28.3 g) Nalgene plastic bottles containing 12 mL of 1% albumin in phosphate buffer plus 40 mg collagenase (type II, Sigma). The bottle was flushed with oxygen and stoppered. The tissues were digested for 1 h at 30°C in a shaking water bath.

The suspension of adipocytes was strained through a mesh (350 μm, metal) and centrifuged at 100 × *g* for 30 s in a polyethylene centrifuge tube. The proteinaceous digest solution in the bottom of the centrifuge tube was removed carefully with a long needle syringe. The adipocytes were resuspended in buffer and centrifuged, and the bottom layer was again removed by syringe. A section of dialysis tubing (Spectra/Por, Los Angeles, Calif.), of 80 cm length and 1.46 cm diameter, was used to separate free fat from adipocytes. The tubing was filled with the phosphate albumin buffer. A clamp was applied about 15 cm from the bottom. The adipocyte suspension was loaded into this empty tubing section, which was sealed by applying a clamp at the bottom end of the tubing. The tubing was suspended vertically for a few seconds, then the clamp on top of the loaded cell suspension was released. As soon as most of the rapidly rising droplets of free fat floated to the top of the tubing, another clamp was applied just below that free fat layer. The adipocytes continued to rise until they collected below the top clamp and could be removed for microscopic observation.

Each isolated cell suspension (5 μL) was applied from underneath a slide into a rectangular framed (1 × 1 cm) cell. This was made by cutting adhesive tape and gluing it to a subbed slide. Both the subbed slide and the cover glass were freshly siliconized before observation. The adipocyte suspension was observed under a Nikon Biological Microscope (Nippon Kogaku K.K., Japan) equipped with a photomicrographic attachment (Microflex, Nikon Model HFX, Japan). Photomicrographs were immediately taken at different locations of the slide.

The diameters of both isolated and histologically revealed (in situ) adipocytes were measured by projecting the slides and reading the adipocyte images. The plots of diameters of adipocyte populations were achieved by measuring at least 500 cells from belly flaps and mesenteric adipose tissue, except for adipocytes isolated from myosepta of white muscle, of which only 180 cells were counted. Because of the polygonal characteristics of adipocytes on the histological sections, the apparent diameter of each adipocyte was measured by averaging two widths of cytoplasmic rim to rim taken at 90°.

Results

The lipid content of whole muscle tissue used for histological studies was 10.82 ± 0.34 and 9.01 ± 0.45% (wet basis, triplicates) for the male and female fish, respectively. The slightly lower lipid content of the female salmon was apparently due to the mobilization of lipids from the muscle tissue to the maturing eggs.

Adipocytes were found in all connective tissues but their size and density varied according to the type of tissue and its location. The connective tissues of the mesentery and belly flaps were characterized by numerous adipocytes, which displayed the same characteristics of cell morphology, size range, and density in both tissues (Figs. 1A and 1B); blood vessels were also clearly visible.

High densities of adipocytes were also present in the myosepta of the lower flank region, but more intercellular fibres or matrix were observed in this type of connective tissue than in the belly flaps (Fig. 2). Adipocytes are polygonal when contiguous to other cells, and their peripheral nuclei were often clearly seen in the sections of belly flaps (Fig. 1B), mesenteric tissue, subcutaneous fat layer (Fig. 3A), myosepta, and adipose fin (Fig. 3B). Most of the adipocytes were found in situ in frozen sections, but in a small number of adipocytes the lipids had moved during sectioning because they were not fixed. However, the cytoplasmic membrane of these adipocytes was fixed and clearly visible.

The muscle tissues were surrounded by a layer of subcutaneous fat, which was more concentrated along the lateral line of the dark muscle, and became much thicker towards the belly flap regions. Adipocytes constituted the major cell type in this layer of subdermal tissue (Fig. 3A). Almost all of the myosepta from the white muscle penetrated the dark muscle layer and passed through to the subdermal fat layer (Zhou et al.

Fig. 1. Light micrographs of adipocytes in (A) mesenteric tissue and (B) belly flaps of Atlantic salmon (frozen sections, oil red O staining) showing a high density of adipocytes, the adipocyte membrane (AM) and nucleus (AN), as well as numerous blood vessels (BV). The light micrographs for both belly flaps and mesenteric tissue were similar in terms of adipocyte distribution. (A) Scale bar = 250 μm. (B) Scale bar = 100 μm.

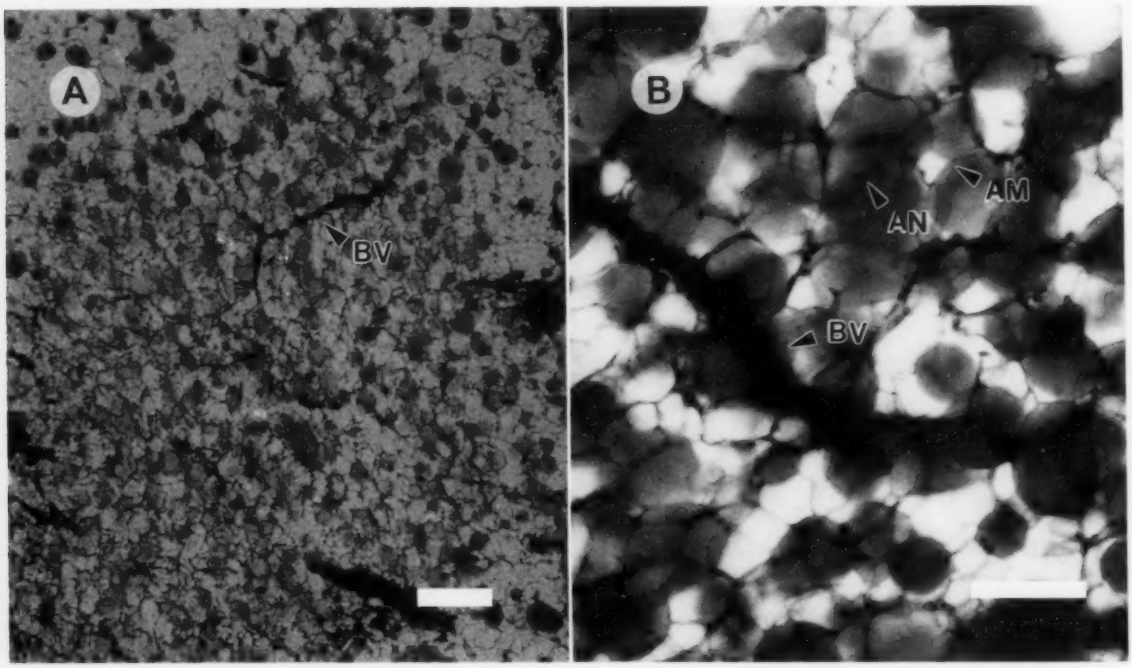
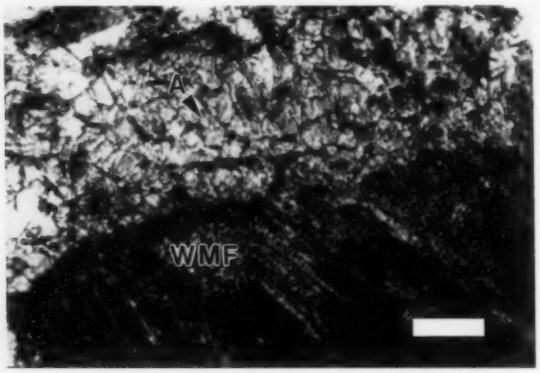


Fig. 2. Light micrograph of myoseptum in lower flank region of Atlantic salmon (frozen section, oil red O staining) showing adipocytes (A) and bands of white muscle fibres (WMF) attached to the myoseptum. Scale bar = 250 μm.



1995). Very few myosepta were isolated solely within the muscle tissue.

Adipocytes in the adipose fin were present mainly in the loose connective tissue of the core area (about one-third to one-fourth of the fin thickness) at a slightly lower density (Fig. 3B) than those in the belly flaps. Single adipocytes were also observed in the dermal layer near the core area of the adipose fin.

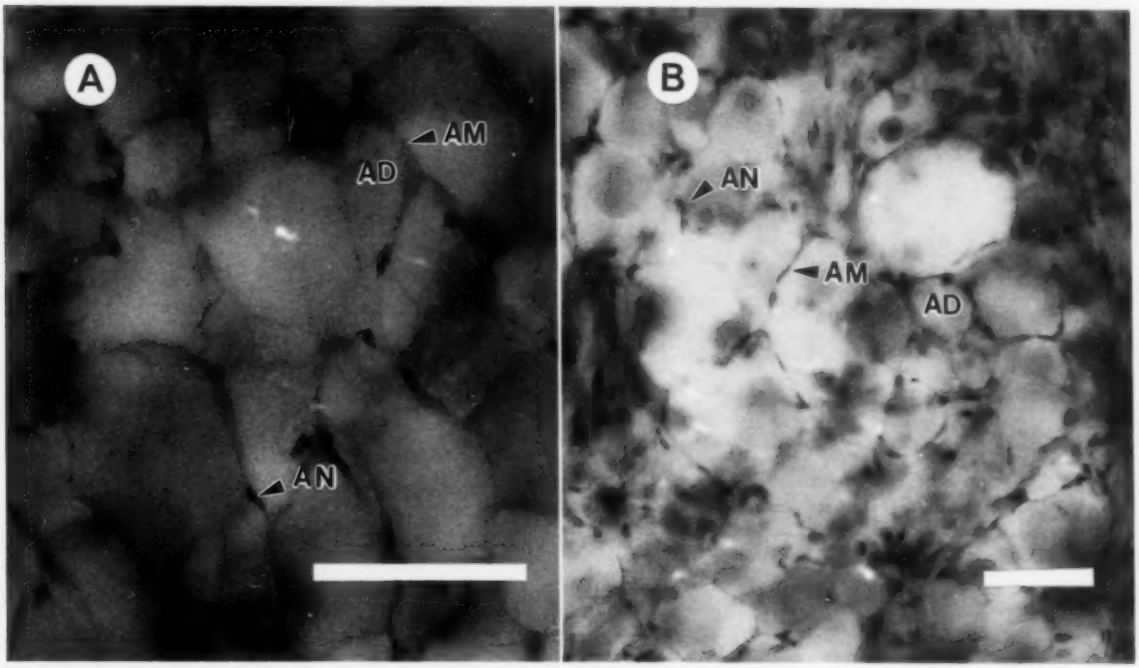
The myoseptum is one of the major connective tissues present in muscle tissue. The myoseptum volume proportions increased sharply from the dorsal region to the belly flaps. There were high proportions of intercellular fibre, ground substances (glycosaminoglycan, Wheater et al. 1979), capillaries, and nonadipocyte cells in the myosepta of the dorsal region (Fig. 4A), especially in the leaner fish. Adipocytes were densely packed in the myosepta, although there were differences in the size range and density of the adipocytes in different regions of the muscle. In the innermost portion of myosepta of the dorsal muscle, adjacent to the visceral cavity, the adipocytes were smaller. The adipocytes were more densely packed and larger closer to the belly flaps.

Adipocytes were also present in the perimysium surrounding bundles of muscle fibres and the endomysium surrounding each individual muscle fibre, although their densities were lower than those in the myosepta. Adipocytes were rarely observed in the endomysium of dorsal white muscle, but they were easily found in the perimysium and endomysium closer to the belly flaps. Lipid droplets were present in the endomysium surrounding each individual dark muscle cell.

No intracellular lipid droplets were observed in white muscle fibres; however, finely dispersed lipid droplets were visible throughout the dark muscle cells. Most of the connective tissue at the junction between dark muscle and white muscle fibres contained a high concentration of lipids; however, some regions were totally devoid of lipids (Fig. 4B).

The adipocytes isolated from belly flaps and mesenteric tissue were spherical (Figs. 5A and 5B). The freed adipocytes

Fig. 3. Light micrographs showing adipocyte distribution in (A) subcutaneous layer and (B) core area of adipose fin of Atlantic salmon (frozen sections, oil red O staining). AD, adipocyte; AM, adipocyte membrane; AN, adipocyte nucleus. Scale bars = 100 μm.



had a strong tendency to form aggregations, especially those from belly flaps. The presence of bi- or multi-locular adipocytes could not be excluded as we were not able to completely distinguish the aggregations from multilocular adipocytes. A portion of the freed adipocytes was broken during the incubation of tissue with collagenase, and thus free lipid droplets were present in every collagenase digest. However, the volume from this damage was usually small in proportion to the amount of isolated adipocytes. Examination of the isolated adipocytes under the microscope showed that flotation of the cell suspension in buffer removed most of the free lipid droplets.

A population of very small adipocytes dominated both the adipocytes observed in situ by histology and those isolated from collagenase treatment (Fig. 6). The two methods of size examination were in full agreement that the major population of adipocytes in both belly flaps and mesenteric tissue was centered around 20-40 μm. The average diameters of adipocytes in belly flaps and mesenteric tissue were 38.9 and 38.4 μm, respectively, from the in situ examination, and 40.5 and 41.2 μm, respectively, from the examination of the isolate. There was no significant difference in the average size of adipocytes between belly flaps and mesenteric adipose tissue. However, the enzymatically freed adipocytes showed a wider size range of 5–140 μm, while the histological sections only displayed an adipocyte size range of 10–90 μm for a similar number of adipocytes. The population of isolated adipocytes from myosepta of white muscle was centered in a smaller size range, with those of diameter 10–30 μm constituting the major cell fraction (Fig. 6). This agrees with the histological observations that adipocytes in the myosepta of the dorsal white muscle are smaller than those in the belly flap region. No significant difference in adipocyte morphology and size distribution was observed in the same tissues from fish of different sizes.

Discussion

Belly flap tissue is the major lipid depot of the muscle tissue in Atlantic salmon and in it the lipid content is up to 10 times higher than that of dorsal white muscle (Ackman et al. 1994). The concentrated packing of adipocytes in myosepta, especially in the belly flaps, supports the observation that this region is the major energy storage site of the fish. Conversely these white muscle connective tissues became very thin in fish depleted in lipids through starvation (S. Zhou, R.G. Ackman, and C. Morrison, unpublished observations), which suggests that they are the major sites for lipid deposition and depletion in Atlantic salmon.

Connective tissues are able to interconnect and nourish other tissues. They carry blood vessels and mediate the exchange of metabolites between tissues and the circulatory system. The blood vessels and comparable lymphatic capillaries are ensheathed by the amorphous ground substance in loose connective tissue. Blood vessels in or adjacent to adipose tissue are abundant because they are the only route for the transportation of lipids towards or from the adipocytes (Cormack 1984).

The difference in the deposition and depletion of lipids in different areas of the muscle tissue might be responsible for the size variation of adipocytes between the dorsal white muscle and the belly flaps. During periods of fasting or active

Fig. 4. Light micrographs of (A) dorsal white muscle and (B) junction area of dark muscle and white muscle of Atlantic salmon (frozen sections, oil red O staining). AD, adipocyte; WMF, white muscle fibre; M, myoseptum; DMF, dark muscle fibre; CWA, connective tissue with adipocytes; CDA, connective tissue devoid of adipocytes. (A) Scale bar = 100 μm. (B) Scale bar = 250 μm.

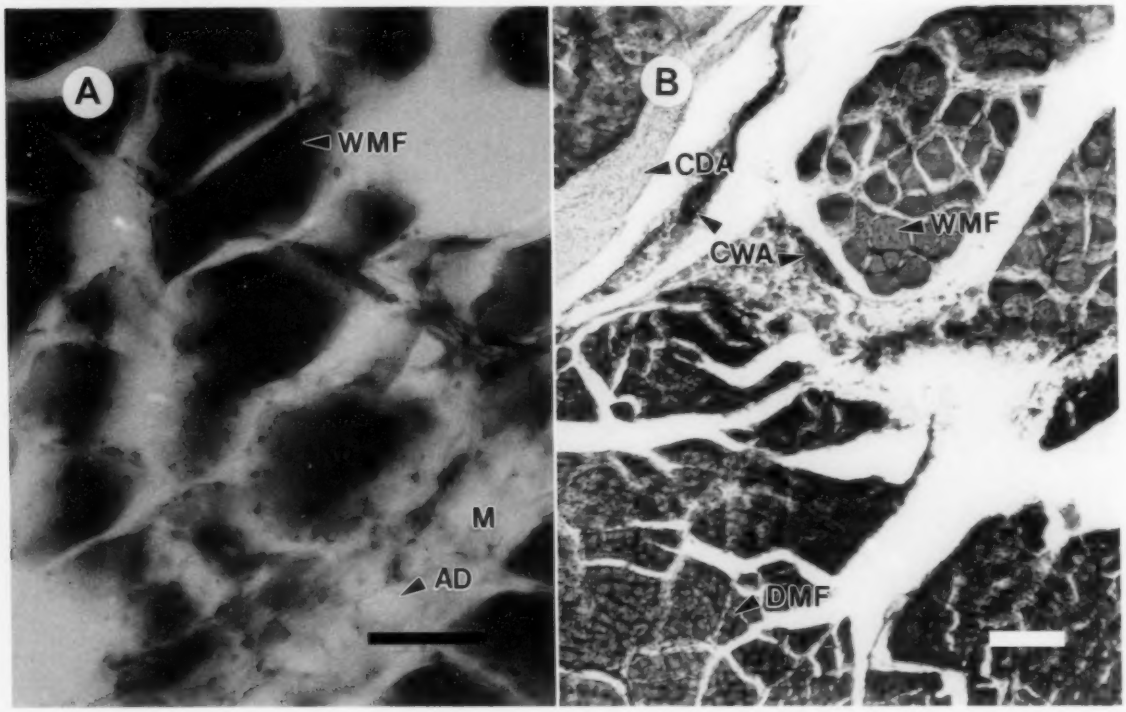
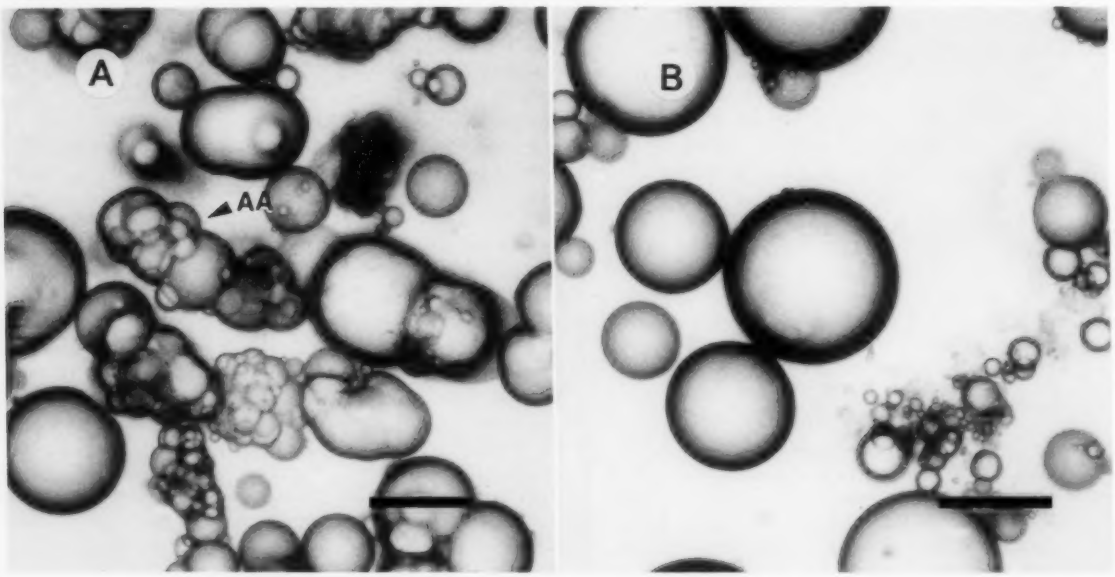


Fig. 5. Light micrographs of adipocytes enzymatically isolated from (A) belly flaps and (B) mesenteric tissue. AA, aggregated adipocytes. Scale bar = 100 μm.



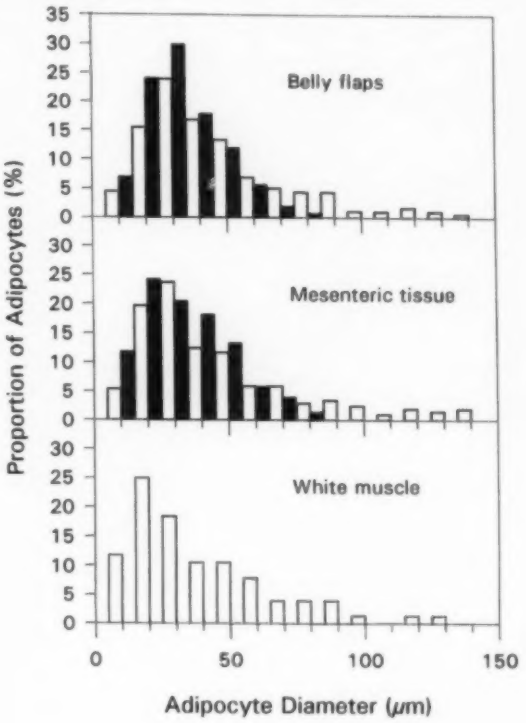
movement of fish, the hormone-sensitive lipase within the adipocytes is activated and fatty acids are hydrolyzed from triglycerides (Bloom and Fawcett 1975). The free fatty acids are then released from the adipocytes, pass through the ground substance by diffusion, and are carried away by capillaries. The diffusion of free fatty acids through the ground substance of connective tissue is probably one of the key steps in controlling the mobilization of lipids in adipocytes, and thus affects the size and density of the adipocyte population. In the first instance, the presence of a lower density of adipocytes, and therefore the relatively higher vascularity around the adipocytes in the myosepta of the dorsal region, favours the absorption of released free fatty acids and thus increases the gradient density of free fatty acids between the blood capillaries and the adipocytes. In the second instance, myosepta in the dorsal white muscle are surrounded by abundant muscle fibres, and the adipocytes in this region are probably used as the immediate source for supplying energy to the adjacent muscle fibres. Both factors lead to the smaller size and lower density of adipocytes present in the dorsal white muscle region.

The lack of lipid inclusions inside white muscle fibres and the presence of few adipocytes in their surrounding connective tissue may also indicate that the main energy source in this muscle tissue comes from glycogen, not from lipids. This agrees with the finding of Nag (1972) that the dark muscle fibres in rainbow trout are rich in lipid and relatively low in glycogen whereas white muscle fibres have little lipid and much glycogen.

Besides Atlantic salmon, intracellular lipid droplets in dark muscle fibres have also been observed in Japanese char (*Salvelinus pluvius*), rainbow trout (*Oncorhynchus mykiss*), horse mackerel (*Trachurus japonicus*), and mackerel (*Scomber japonicus*) (Shindo et al. 1986). These observations support the hypothesis that dark muscle is used for long and sustained swimming movement. Lipids would provide the major energy source for this activity because the proportion of mitochondria in dark muscle is much higher than that in white muscle (Nag 1972) and these droplets are most probably surrounded by mitochondria where energy is produced from lipid oxidation. Fauconneau et al. (1993) also remarked on the depot fats associated with dark muscle fibres of rainbow trout. Electron microscopy observations on dark muscle fibres of Antarctic notothenioid fish revealed that large numbers of lipid droplets were intimately surrounded by mitochondria (Lin et al. 1974). A rich lipid presence has been observed in the endomysium of dark muscle cells, which ensures a continuous supply of intracellular lipids for release and oxidation during long and sustained swimming. The depleted lipid vacancy in the endomysium would, in turn, be supplied from the adipocytes of the nearest myosepta. The presence of both intra- and inter-cellular lipids and the high proportion of myosepta (Zhou et al. 1995) are the reasons that the lipid content of dark muscle is higher than that of white muscle (Ackman et al. 1994).

There are few reported morphological studies on the size distribution of adipocytes in fish. Weisel (1968) investigated the adipose fin of *Thymallus arcticus* and showed the size range of lipid droplets to be 10–50 μm in diameter. A population of very small adipocytes, found in Atlantic salmon during our studies by histological observation and enzymatic digestion, was also reported in rainbow trout (Fauconneau et al. 1993). The dominant presence of small (<50 μm) adipocytes in Atlantic salmon differs from the normal uniform adipocyte population found in human and other mammalian animals, which is usually centered around 80 μm (Ashwell et al. 1976; DeMartinis and Francendese 1982; Meignen et al. 1990). Very small adipocytes (8–35 μm) have been observed in some mammals, but in a very low proportion (DeMartinis and Francendese 1982; DeMartinis 1985).

Fig. 6. Size distributions (10-μm classes) of adipocytes measured in situ in histological sections (solid bars) and those isolated through collagenase digestion (open bars) from Atlantic salmon belly flaps, mesenteric tissue, and myosepta of white muscle (average from two fish for each procedure). Adipocyte size distributions in white muscle were measured only through the enzymatic isolation procedures.



The presence of small adipocytes in Atlantic salmon may well be a characteristic feature of adipocyte populations in this species. According to our unpublished data, the daily diet intakes of Atlantic salmon were totally dependent on the water temperature. This is, to some extent, similar to the findings for hibernating animals, which completely stop eating during their hibernating period and in which brown adipocytes play an important role in heat production during the process of arousal from hibernation (Joel 1965). The small adipocytes in Atlantic salmon might significantly contribute to the maintenance of an energy supply at low temperatures, as they have relatively more mitochondria associated with the main lipid body. For the same reason, they may be the most active sites for lipid deposition and depletion when excess diet energy must be stored or when they are fasting. Fauconneau et al. (1993)

suggested that the small adipocytes in fish are a developmental stage during which additional fat is being stored.

Aggregation of isolated adipocytes was not the case for the adipocytes isolated from humans and other mammals (Lorch and Rentsch 1969; Smith et al. 1972). The isolation of adipocytes from belly flaps and myosepta of white muscle is different from that from mesenteric tissue because of the absence of muscle fibres in the latter tissue. The aggregation of adipocytes isolated from belly flaps and myosepta of white muscle was postulated to be due to the presence of phospholipids released from the membranes of muscle fibres, or of protein fragments produced during the digestion period. These compounds possess amphoteric characteristics and have a strong affinity with the membranes of adipocytes. The aggregated adipocytes were not split up through the subsequent washing and flotation procedures. The lower incidence of adipocyte aggregation from mesenteric tissue was apparently due to the low concentration of protein fragments and phospholipids in the digest.

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References

Ackman, R.G. 1989. Nutritional composition of fats in seafoods. Prog. Food Nutr. Sci. **13**: 161–241.

Ackman, R.G., Zhou, S., and Heras, H. 1994. Effect of different lipid levels on the uptake and depuration of petroleum hydrocarbons by Atlantic salmon. *In* Proceedings of Third Joint Conference of the Atlantic Fisheries Technology Society and the Tropical and Subtropical Fisheries Society, Williamsburg, Va., August 29 – September 1, 1993. Florida Sea Grant College Program, No. SGR-114, Gainesville, Fla. pp. 388–397.

Ashwell, M., Priest, P., Bondoux, M., Sowter, C., and McPherson, C.K. 1976. Human fat cell sizing: a quick, simple method. J. Lipid Res. **17**: 190–192.

Bligh, E.G., and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**: 911–917.

Bloom, W., and Fawcett, D.W. 1975. A textbook of histology. 10th ed. W.B. Saunders Company, Philadelphia, Pa.

Cormack, D.H. 1984. Introduction to histology. J.B. Lippincott Company, Philadelphia, Pa.

DeMartinis, F.D. 1985. Very small fat cell populations determined by a modified osmium tetroxide-urea method. Am. J. Physiol. **249**: C89–C96.

DeMartinis, F.D., and Francendese, A. 1982. Very small fat cell populations: mammalian occurrence and effect of age. J. Lipid Res. **23**: 1107–1120.

Eastman, J.T., and DeVries, A.L. 1982. Buoyancy studies of notothenioid fishes in McMurdo Sound, Antarctica. Copeia, 1982: 385–393.

Fauconneau, B., Chmaitilly, J., André, S., Cardinal, M., Cornet, J., Vallet, J.L., Dumont, J.P., and Laroche, M. 1993. Characteristics of rainbow trout flesh. I. Chemical composition and cellularity of muscle and adipose tissues. Sci. Aliment. **13**: 173–187.

Humason, G.L. 1979. Animal tissue techniques. 4th ed. W.H. Freeman and Company, San Francisco, Calif.

Joel, C.D. 1965. The physiological role of brown adipose tissue. *In* Handbook of physiology, section 5. Adipose tissue. *Edited by* A.E. Renold and G.F. Cahill. American Physiology Society, Washington, D.C. pp. 59–85.

Johnson, P.R., and Greenwood, M.R.C. 1983. The adipose tissue. *In* Histology, cell and tissue biology. 5th ed. *Edited by* L. Weiss. Elsevier Science Publishing Co., Inc., New York. pp. 178–199.

Lin, Y., Dobbs, G.H., and Devries, A.L. 1974. Oxygen consumption and lipid content in red and white muscles of Antarctic fishes. J. Exp. Zool. **189**: 379–386.

Lorch, E., and Rentsch, G. 1969. A simple method for staining and counting isolated adipose tissue fat cells. Diabetologia, **5**: 356–357.

Meignen, J.M., Desnuelle, C., and Boyer, J. 1990. A simple device for the optimized isolation of fat cells. Lipids, **25**: 412–417.

Nag, A.C. 1972. Ultrastructure and adenosine triphosphatase activity of red and white muscle fibres of the caudal region of a fish, *Salmo gairdneri*. J. Cell Biol. **55**: 42–57.

Polvi, S.M., and Ackman, R.G. 1992. Atlantic salmon (*Salmo salar*) muscle lipids and their response to alternative dietary fatty acid resources. J. Agric. Food Chem. **40**: 1001–1007.

Robinson, J.S., and Mead, J.F. 1973. Lipid absorption and deposition in rainbow trout (*Salmo gairdneri*). Can. J. Biochem. **51**: 1050–1058.

Sheridan, M.A., Allen, W.V., and Kerstetter, T.H. 1983. Seasonal variations in the lipid composition of the steelhead trout, *Salmo gairdneri* Richardson, associated with the parr–smolt transformation. J. Fish Biol. **23**: 125–134.

Shindo, K., Tsuchiya, T., and Matsumoto, J.J. 1986. Histological study on white and dark muscles of various fishes. Nippon Suisan Gakkaishi, **52**: 1377–1399.

Sinnett-Smith, P.A., and Waddington, D. 1992. Size distribution of adipocytes and variation in adipocyte number in lines of mice selected for high or low body fat. Comp. Biochem. Physiol. A Comp. Physiol. **102**: 573–578.

Smith, U., Sjöström, L., and Björntorp, P. 1972. Comparison of two methods for determining human adipose cell size. J. Lipid Res. **13**: 822–824.

Weisel, G.F. 1968. The salmonoid adipose fin. Copeia, 1968: 626–627.

Wheater, P.R., Burkitt, H.G., and Daniels,V.G. 1979. Functional histology, a text and color atlas. Churchill Livingstone, Edinburgh, U.K.

Yamada, J. 1972. Histochemical observation of fish muscle. II. Occurrence and status of lipids and muscle texture of tunas. Bull. Tokai Reg. Fish. Res. Lab. No. 72. pp. 35–42.

Yamada, J., and Nakamura, S. 1964. Histological observation of fish muscle. I. Distribution of fat in important food fish. Bull. Tokai Reg. Fish. Res. Lab. No. 39. pp. 21–28.

Zhou, S., Ackman, R.G., and Morrison, C. 1995. Storage of lipids in the myosepta of Atlantic salmon (*Salmo salar*). Fish Physiol. Biochem. **14**: 171–179.

Genetic variation within *Pseudoterranova decipiens* (Nematoda: Ascaridoidea) from Canadian Atlantic marine fishes and seals: characterization by RFLP analysis of genomic DNA

J. Brattey and W.S. Davidson

Abstract: Genetic variation in the ribosomal RNA gene complex (rDNA) of 181 individual *Pseudoterranova decipiens* from three seal species and six marine fish species collected widely throughout Atlantic Canada was investigated by restriction endonuclease digestion followed by Southern blotting using a heterologous cloned rDNA repeating unit as a probe. Five enzymes (*Eco*RI, *Hae*II, *Hpa*I, *Pst*I, *Pvu*II) yielded informative restriction fragment length polymorphisms and the results, which corroborate previous studies of allozyme variation, indicate two species with no evidence of hybridization or introgression between them: one species (= *P. decipiens* C) infects bearded seals, *Erignathus barbatus*, and is characterized by six unique restriction fragments; the other (= *P. decipiens* B) infects both harbour seals, *Phoca vitulina*, and grey seals, *Halichoerus grypus*, and is characterized by 10 unique restriction fragments. Larvae from most areas throughout Atlantic Canada showed restriction fragment length polymorphisms characteristic of *P. decipiens* B, but some specimens collected from flatfishes off Labrador were *P. decipiens* C. Restriction fragment patterns also revealed polymorphism, both within and between species, and among ribosomal repeat units within individuals, particularly within *P. decipiens* B. The genetic polymorphisms observed in *P. decipiens* from grey and harbour seals were similar, suggesting interbreeding among nematodes from these two pinniped species.

Résumé : Pour l'étude de la variation génétique du complexe des gènes d'ARN ribosomaux (rDNA) de 181 spécimens de *Pseudoterranova decipiens* provenant de trois espèces de phoques et de six espèces de poissons marins recueillis sur une vaste étendue du Canada atlantique, on a utilisé la technique de la digestion par l'endonucléase de restriction, suivie par transfert de Southern, en utilisant comme sonde une unité répétitive de rDNA hétérologue clonée. Cinq enzymes (*Eco*RI, *Hae*II, *Hpa*I, *Pst*I, *Pvu*II) ont permis d'obtenir des informations utiles sur les polymorphismes associés à la longueur de fragments de restriction, et les résultats, qui corroborent des études antérieures de variations des allozymes, mettent en évidence deux espèces ne présentant aucun signe d'hybridation ou d'introgression. L'une (*P. decipiens* C) infecte le phoque barbu, *Erignathus barbatus*, et est caractérisée par six fragments de restriction spécifiques; l'autre (*P. decipiens* B) infecte le phoque commun, *Phoca vitulina*, et le phoque gris, *Halichoerus grypus*, et est caractérisée par 10 fragments de restriction spécifiques. Les larves de la plupart des régions du Canada atlantique présentaient des valeurs de polymorphisme associé à la longueur de fragments de restriction caractéristiques de *P. decipiens* B, mais certains spécimens prélevés chez des poissons plats au large du Labrador étaient des *P. decipiens* C. Les profils des fragments de restriction ont également mis en évidence le polymorphisme, tant à l'intérieur des espèces qu'entre celles-ci, et entre les unités ribosomales répétitives chez des individus, et notamment chez *P. decipiens* B. Les polymorphismes génétiques observés chez les *P. decipiens* des phoques gris et des phoques communs étaient semblables, ce qui suggère qu'il y a des croisements entre les nématodes de ces deux espèces de pinnipèdes.
[Traduit par la Rédaction]

Introduction

Third-stage larvae of the parasitic nematode *Pseudoterranova decipiens* (sealworm) commonly infect marine fishes in cool temperate and subarctic oceans where pinniped definitive hosts are common. In Atlantic Canada, third stage larvae of *P. decipiens* infect a broad spectrum of demersal and benthic marine fishes (Templeman et al. 1957; McClelland et al. 1990). The larval nematodes are unsightly and can infect humans (Margolis 1977; Nagano 1989) and their removal from fish flesh during processing is an expensive problem for the Canadian Atlantic fishing industry (Malouf 1986). Increases

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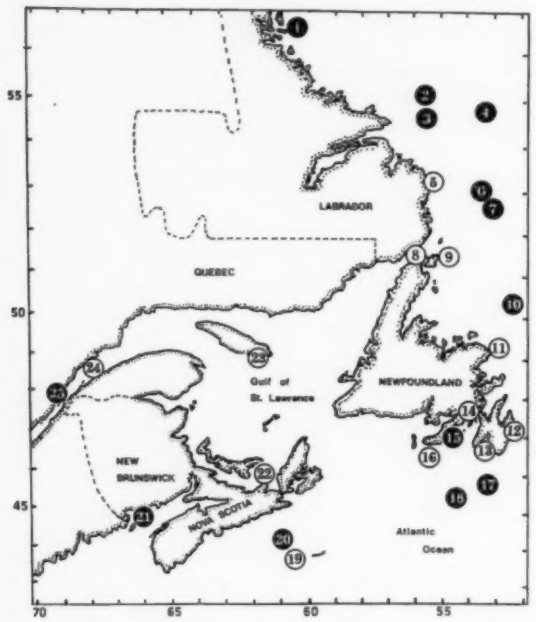
Fig. 1. Map of Atlantic Canada showing numbered collection sites for hosts of *Pseudoterranova decipiens* (black numbers in open circles for seals, white numbers in solid circles for fishes). See Table 1 for further details.



in the abundance of larval *P. decipiens* in several fisheries in Atlantic Canada (McClelland et al. 1983*a*, 1983*b*, 1985; Brattey et al. 1990) have prompted extensive research on various aspects of the biology of these nematodes (Bowen 1990).

Recently, studies of allozyme variation revealed that *P. decipiens* in the North Atlantic and adjacent areas consists of a complex of morphologically similar but reproductively isolated species with different preferred hosts and geographic distributions (Paggi et al. 1991; Brattey and Stenson 1993). In the northeast Atlantic and Norwegian Sea two sympatric species exist, designated *P. decipiens* A and *P. decipiens* B; adults of the former are most common in the grey seal, *Halichoerus grypus*, whereas adults of the latter predominate in the harbour seal, *Phoca vitulina*. In Atlantic Canada, *P. decipiens* A is apparently absent and *P. decipiens* B infects both grey and harbour seals, and occasionally hooded seals, *Cystophora cristata*. Adults of a third species, *P. decipiens* C, are found in the bearded seal, *Erignathus barbatus*, and are widely distributed, occurring at higher latitudes in both the northwest Atlantic and Barents Sea.

Appleton and Burt (1991) suggested that *P. decipiens* B in Atlantic Canada could be further subdivided into two variants on the basis of isoelectric focusing of soluble protein extracts and unpublished evidence from infectivity studies. These variants occurred sympatrically in various species of marine fish with type I predominating in the lower Bay of Fundy and type II more common in the Gulf of St. Lawrence. In light of these contradictory findings, we investigated genetic variation within *P. decipiens* from Atlantic Canada using a different approach.

We reexamined genetic variation within *P. decipiens* by analysis of restriction fragment length polymorphisms (RFLPs) in repetitive rDNA sequences of individual nematodes. RFLPs and allozymes are inherited as simple Mendelian codominant markers and both therefore give more rigorous evidence of genetic segregation than phenotypic characters such as soluble protein profiles, which are prone to nongenetic variation. Furthermore, RFLPs may provide a more sensitive measure of genetic subdivision than allozymes (Karl and Avise 1992). RFLPs have been used to differentiate between closely related parasitic nematodes from various taxa (Curran et al. 1985; Klassen et al. 1986; Cameron et al. 1988; Adamson et al. 1992; Grant and Whitington 1994) including marine ascaridoids (Sugane et al. 1989; Matsuura et al. 1992). Our objectives were (*i*) to determine if there were any RFLPs in rDNA of the *P. decipiens* species complex in Atlantic Canada, and (*ii*) to attempt to resolve the opposing views of Paggi et al. (1991) and Appleton and Burt (1991). We investigated genetic variation in the rDNA of individual nematodes from various hosts and localities by restriction endonuclease digestion followed by Southern blotting using a cloned heterologous repeating rDNA unit as a probe.

Materials and methods

Collection of samples

Live adult nematodes were collected from the stomach of seals as described by Brattey and Stenson (1993), washed several times in saline solution, and individually frozen (–60°C) in labelled microcentrifuge tubes. Fish were collected during Department of Fisheries and Oceans research cruises using bottom trawls and initially stored whole on ice for up to 7 days. Live third-stage larvae (L3s) of *P. decipiens* were recovered from flesh and viscera by visual inspection, or by digesting flesh and viscera separately in warm (37°C) pepsin-HCl solution (1 g of 1 : 10 000 pepsin and 10 mL of concentrated HCl per litre) for up to 6 h; live L3s were recovered, washed, and frozen for storage as described above for adult nematodes. A total of 181 specimens of *P. decipiens* was used in the study; these were obtained from three seal species and six fish species collected widely across Atlantic Canada (Fig. 1, Table 1). Small (5 g) pieces of muscle tissue were also taken from seals and frozen (–60°C) for storage.

Extraction of DNA

Individual nematodes, or small pieces of seal muscle tissue (100 mg), were briefly thawed and ground in Ten Broeck glass homogenizers and nucleic acids purified using the phenol : chloroform : isoamyl alcohol extraction procedure described by Maniatis et al. (1982), except that ethylene glycol rather than ethanol was used in the final precipitation step. Pelleted nucleic acids from individual specimens were resuspended in 65–300 mL of TE (10 mM Tris, 1 mM EDTA (ethylenediaminetetraacetic acid), pH 8.0) and stored at 4°C.

Restriction enzymes

The following restriction enzymes were used to digest aliquots of 0.25–0.5 µg of DNA from individual nematodes or seals: *Eco*RI, *Hae*II, *Hae*III, *Hin*dIII, *Hin*fI, *Hpa*I, *Pst*I, *Pvu*II, *Taq*I, *Xba*I, and *Xho*I, with digestions set up following the instructions supplied by the manufacturer (Pharmacia for *Eco*RI, *Pst*I, *Pvu*II, and *Taq*I; Boehringer-Mannheim for the remainder).

Electrophoresis and Southern blotting

DNA fragments were separated in 1.0% agarose gels (20 cm long) using 1× TBE buffer (89 mM Tris, 89 mM boric acid, 10 mM EDTA, pH 8.3). Gels were run for 18–24 h at 4–5 V/cm. Lambda DNA cut

Table 1. Sampling details for specimens of *Pseudoterranova decipiens* used in the study.

Host species	Host number	Collection date	Collection site[a] (code)	Number of specimens
Seals				
Erignathus barbatus (bearded seal)	1	Jan. 1993	Greenspond, Nfld. (11)	6
	2	Jan. 1993	Greenspond, Nfld. (11)	6
	3	May 1993	Noddy Bay, Nfld. (9)	1
	4	May 1993	Noddy Bay, Nfld. (9)	6
	5	May 1993	Noddy Bay, Nfld. (9)	3
	6	April 1994	Straitsview, Nfld. (8)	4
	7	May 1994	Black Tickle, Labrador (5)	14
Halichoerus grypus (grey seal)	1	Oct. 1988	Anticosti Island, Que. (23)	7
	2	June 1989	Isle aux Lievres, Que. (24)	1
	3	May 1989	Sable Island, N.S. (19)	4
	4	July 1990	Cape Broyle, Nfld. (12)	1
	5	Aug. 1990	Garden Cove, Nfld. (14)	3
	6	Jan. 1994	SE Gulf of St. Lawrence (22)	4
	7	Jan. 1994	SE Gulf of St. Lawrence (22)	33
Phoca vitulina (harbour seal)	1	Aug. 1990	St. Mary's Bay, Nfld. (13)	1
	2	Aug. 1990	St. Mary's Bay, Nfld. (13)	28
	3	Sept. 1990	Point May, Nfld. (16)	2
	4	Sept. 1990	Point May, Nfld. (16)	1
	5	Sept. 1990	Point May, Nfld. (16)	1
	6	Sept. 1990	Point May, Nfld. (16)	10
	7	Sept. 1990	Point May, Nfld. (16)	3
Fishes				
Gadus morhua (Atlantic cod)	1	April 1991	South coast, Nfld. (18)	1
	2	April 1993	Scotian shelf, N.S. (20)	1
	3–5	Jan. 1994	Placentia Bay, Nfld. (15)	5
Hemitripterus americanus (sea raven)	1, 2	Sept. 1992	Grand Manan, N.B. (21)	13
Myoxocephalus scorpius (shorthorn sculpin)	1–3	Jan. 1994	Northeast coast, Nfld. (10)	4
	4–7	April 1994	South coast, Nfld. (17)	5
Hippoglossus hippoglossus (Atlantic halibut)	1	July 1991	Labrador (6)	1
	1	Aug. 1992	Labrador (7)	1
Hippoglossoides platessoides (American plaice)	1, 2	July 1990	Labrador (1)	2
	3	July 1992	Labrador (2)	1
	4–8	July 1992	Labrador (3)	5
Reinhardtius hippoglossoides (turbot)	1	Feb. 1991	Saguenay, Que. (25)	1
	2	Nov. 1991	Labrador (4)	2

[a]Site codes are given in parentheses. The locations of coded collection sites are indicated in Fig. 1.

with *Hin*dIII was used as a size marker. Digests of seal DNA were run alongside nematode DNA as controls to determine whether the gut tissues of nematodes contained host DNA that might compromise interpretation of the results. To check that digestion was complete, gels were soaked for 30 min in 1× TBE buffer containing 0.5 µg/mL ethidium bromide and viewed under short-wave ultraviolet light. The gels were soaked for 1 h in denaturing solution (0.5 M NaOH, 1.5 M NaCl) and the DNA was transferred to 0.45-µm Biodyne B nylon membrane following the instructions of the manufacturer (Pall Biosupport Div., East Lansing, N.Y.) using a modification of the method of Southern (1975).

Ribosomal DNA probe

The probe, p*Dm*238, contains an 11.0 kilo base pair (kbp) *Eco*RI fragment containing the entire rDNA repeat of *Drosophila melanogaster* cloned into the plasmid pBR322; it was kindly provided by J. Hancock, Genetics Department, Cambridge University, U.K.

Hybridization

Membranes were incubated at 37°C for 4–6 h with 10 mL of prehybridizing solution (50% formamide, 6× SSC (1× SSC is 0.15 M NaCl, 0.015 M trisodium citrate), 5× Denhardt's (1× Denhardt's contains 0.02% bovine serum albumen, 0.02% Ficoll, and 0.02% polyvinyl pyrrolidone), 50 mM phosphate buffer, pH 6.8, and 0.1% SDS). The probe was labelled with [α-^{32}P]dCTP using a kit (Mega-Prime) following the instructions of the manufacturer (Amersham, Inc.). Each membrane was hybridized overnight at 37°C with 10 mL of fresh prehybridizing solution and approximately 2×10^6 dpm of probe. Membranes were washed twice for 10 min at room temperature in 2× SSC, 0.1% SDS, and twice for 10 min at 37°C in 1× SSC, 0.1% SDS, before being blotted dry and placed in exposure cassettes with X-ray film and intensifying screens. Films were developed after 7 days exposure at –70°C.

Results

Preliminary screening

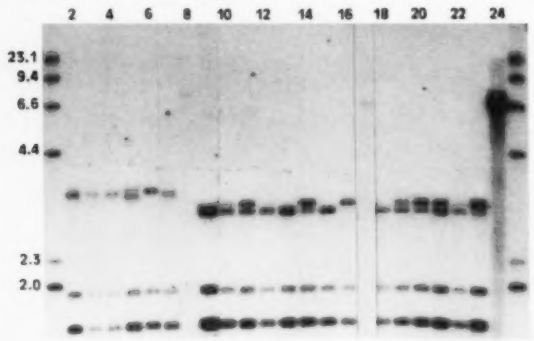
Initially DNA prepared from adult nematodes from bearded, grey, and harbour seals was digested with individual restriction

Table 2. Approximate sizes (in kilo base pairs) of fragments generated by digesting genomic DNA from *Pseudoterranova decipiens* with various restriction enzymes and observed after Southern blotting and hybridization with a ^{32}P-labelled rDNA repeat (p*Dm*238).

Fragment	Restriction enzyme										
	*Eco*RI	*Hae*II	*Hae*III	*Hin*dIII	*Hin*fI	*Hpa*I	*Pst*I	*Pvu*II	*Taq*I	*Xba*I	*Xho*I
A	3.5[a]	3.7	<0.8[b]	23.1	<0.6[b]	7.9	6.2	5.0	<0.7[b]	7.1	23.1
B	3.3	2.0[a]	—	—	—	5.8	6.0	4.9	—	—	—
C	3.2	1.7	—	—	—	5.5	1.5	4.8	—	—	—
D	1.9	1.6	—	—	—	1.9	—	2.9	—	—	—
E	1.6	—	—	—	—	1.8	—	—	—	—	—
F	0.6	—	—	—	—	—	—	—	—	—	—

[a]Appeared as 1–3 tightly spaced bands of variable intensity.
[b]Appeared as 4–8 faint bands that were poorly resolved.

Fig. 2. Autoradiograph of adult *Pseudoterranova decipiens* and seal genomic DNA digested with *Eco*RI, Southern blotted, and probed with a ^{32}P-labelled rDNA repeat (p*Dm*238). Each lane represents DNA from a different individual nematode or seal. Lanes 2–7, *P. decipiens* from *Erignathus barbatus*; lane 8, *E. barbatus* DNA; lanes 9–16, *P. decipiens* from *Halichoerus grypus*; lane 17, *H. grypus* DNA; lanes 18–23, *P. decipiens* from *Phoca vitulina*; lane 24, *P. vitulina* DNA. Lanes 1 and 25 are ^{32}P-labelled λ DNA cut with *Hin*dIII and numbers are fragment sizes in kilo base pairs.



enzymes and subjected to Southern blot analysis using the labelled probe. Five of the enzymes (*Eco*RI, *Hae*II, *Hpa*I, *Pst*I, and *Pvu*II) produced multiple fragments of sizes ranging from 0.6 to 7.9 kbp (Table 2), although fragment A produced by *Eco*RI and fragment B produced by *Hae*II occasionally appeared as fragments of slightly different size and staining intensity. Among the remaining enzymes, *Xba*I produced one large fragment (7.1 kbp), whereas on *Hin*dIII and *Xho*I blots the probe apparently hybridized only to undigested DNA (or digested DNA with a size greater than 23 kbp). The remaining enzymes (*Hae*III, *Hin*fI, and *Taq*I) produced groups of 4–8 small fragments (<0.6 kbp) that were poorly resolved and faint on the autoradiographs. Hence, the enzymes *Eco*RI, *Hae*II, *Hpa*I, *Pst*I, and *Pvu*II produced the most informative restriction fragment patterns, and these enzymes were used throughout the remainder of the study. Duplicate runs of the same samples were also conducted and these confirmed that fragment patterns and relative staining intensities of fragments were reproducible.

Interspecific polymorphism

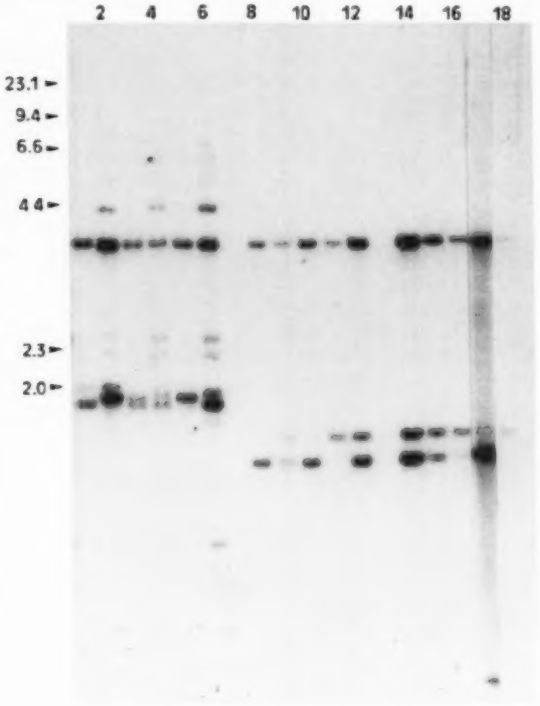
The preliminary screening also revealed several RFLPs among adult nematodes from different seal species. To determine the extent of variation between and within nematodes from different hosts, we digested DNAs from adult nematodes and prepared blots using several specimens from bearded, grey, and harbour seals. A total of 22 restriction fragments was generated by the five informative enzymes (Table 2). The fragment patterns revealed that adult nematodes from seals could be classified into two distinct groups. One group, which occurred only in bearded seals (equivalent to *P. decipiens* C of Paggi et al. 1991), was characterized by the following six restriction fragments: a 3.5-kbp *Eco*RI fragment (Fig. 2); a 2.0-kbp *Hae*II fragment (Fig. 3); 5.8- and 1.8-kbp *Hpa*I fragments (Fig. 4); a 6.2-kbp *Pst*I fragment (Fig. 5); and a 5.0-kbp *Pvu*II fragment (data not shown). The other group, which occurred in grey and harbour seals (equivalent to *P. decipiens* B of Paggi et al. 1991), was characterized by the following 10 restriction fragments: 3.3- and 3.2-kbp *Eco*RI fragments (Fig. 2); 1.7- and 1.6-kbp *Hae*II fragments (Fig. 3); 7.7-, 5.5-, and 1.9-kbp *Hpa*I fragments (Fig. 4); a 6.0-kbp *Pst*I fragment (Fig. 5); and 4.9- and 4.8-kbp *Pvu*II fragments (data not shown). The remaining six fragments were common to all nematode specimens, except for a 1.9-kbp *Hpa*I fragment that was not observed in two specimens from a grey seal (lanes 16 and 17, Fig. 4).

Inspection of the autoradiographs also revealed that the probe either did not hybridize with restriction digests of DNAs from any of the seal species (lanes 7, 13, and 19 in Fig. 3; lanes 8 and 16 in Fig. 5), or produced fragments that differed markedly in size from those of nematode DNAs (lanes 7, 17, and 24 in Fig. 2; lane 25 in Fig. 5). This finding confirms that none of the nematode samples were contaminated with detectable amounts of DNA of host origin.

Intraspecific polymorphism

Genetic polymorphism was also found within *P. decipiens* B and within *P. decipiens* C, but no shared interspecific polymorphisms were detected. Specimens of *P. decipiens* C from bearded seals varied with respect to both the length and number of fragments that appeared at approximately 3.4–3.5 kbp following digestion with *Eco*RI (Fig. 2) and at approximately 1.9–2.0 kbp following digestion with *Hae*II (Fig. 3). Intraspecific polymorphism was more extensive within *P. decipiens* B, where single digestions with *Eco*RI, *Hae*II, *Hpa*I, and *Pvu*II all revealed extensive polymorphism among individuals (Figs. 2–5). The polymorphisms revealed

Fig. 3. Autoradiograph of adult *Pseudoterranova decipiens* and seal genomic DNA digested with *Hae*II and visualized as in Fig. 2. Each lane represents DNA from a different individual nematode or seal. Lanes 1–6, *P. decipiens* from *Erignathus barbatus*; lane 7, *E. barbatus* DNA; lanes 8–12, *P. decipiens* from *Halichoerus grypus*; lane 13, *H. grypus* DNA; lanes 14–18, *P. decipiens* from *Phoca vitulina*; lane 19, *P. vitulina* DNA. Arrows indicate the position of size standards (λ DNA cut with *Hin*dIII) and values are in kilo base pairs.



by three of these enzymes (*Eco*RI, *Hae*II, and *Pvu*II) shared three characteristics: (*i*) they were visualized as one or two fragments that differed in size by 0.1 kbp; (*ii*) specimens that showed both fragments (i.e., restriction fragment type BCDEF in *Eco*RI digests) occurred much more frequently in the population than individuals with only one of the polymorphic fragments (i.e., BDEF or CDEF; Table 3); (*iii*) the fragment patterns revealed by these restriction enzymes suggest that they were revealing the same polymorphism. Initially, we pooled the frequencies of individuals showing only one of the two polymorphic fragments and conducted tests of association between one- and two-fragment polymorphisms revealed by different pairs of restriction enzymes. Multi-way tests of association were not possible because too few nematodes were screened with all three enzymes. Significant associations were found between *Eco*RI and *Hae*II (Fisher's exact test, $P < 0.05$, $N = 22$), *Eco*RI and *Pvu*II ($P < 0.001$, $N = 28$), and *Hae*II and *Pvu*II ($P < 0.001$, $N = 30$). In each comparison, the frequency of individuals that possessed both polymorphic fragments with both enzymes was much higher than expected. We interpret these results as indicating two types of rDNA repeat units that differ by the insertion or deletion of 100 base pairs between

Fig. 4. Autoradiograph of adult *Pseudoterranova decipiens* genomic DNA digested with *Hpa*I and visualized as in Fig. 2. Each lane represents DNA from a different individual nematode. Lanes 2–10, *P. decipiens* from *Erignathus barbatus*; lanes 11–18, *P. decipiens* from *Halichoerus grypus*; lanes 19–26, *P. decipiens* from *Phoca vitulina*. Lanes 1 and 27 are ^{32}P-labelled λ DNA cut with *Hin*dIII and numbers are fragment sizes in kilo base pairs.

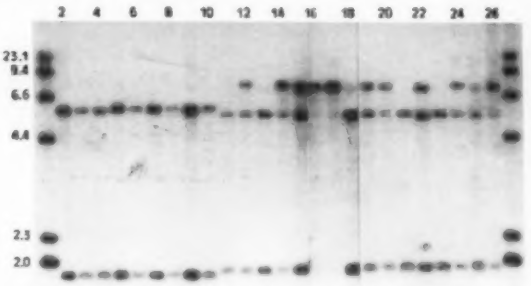
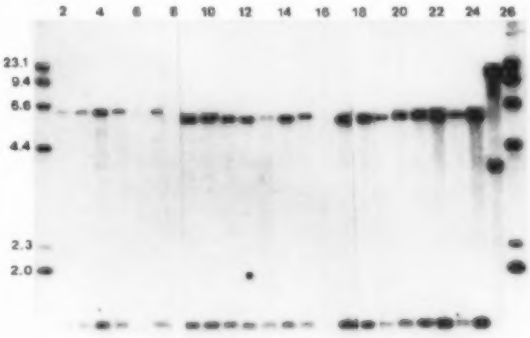


Fig. 5. Autoradiograph of adult *Pseudoterranova decipiens* and seal genomic DNA digested with *Pst*I and visualized as in Fig. 2. Each lane represents DNA from a different individual nematode or seal. Lanes 2–7, *P. decipiens* from *Erignathus barbatus*; lane 8, *E. barbatus* DNA; lanes 9–15, *P. decipiens* from *Halichoerus grypus*; lane 16, *H. grypus* DNA; lanes 17–24 *P. decipiens* from *Phoca vitulina*; lane 25, *P. vitulina* DNA. Lanes 1 and 26 are ^{32}P-labelled λ DNA cut with *Hin*dIII and numbers are fragment sizes in kilo base pairs.piens and seal genomic DNA digested with *Eco*RI, Southern blotted, and probed with a ^{32}P-labelled rDNA repeat (p*Dm*238). Each lane represents DNA from a different individual nematode or seal. Lanes 2–7, *P. decipiens* from *Erignathus barbatus*; lane 8, *E.barbatus* DNA; lanes 9–16, *P. decipiens* from *Halichoerus grypus*; lane 17, *H. grypus* DNA; lanes 18–23, *P. decipiens* from *Phoca vitulina*; lane 24, *P. vitulina* DNA. Lanes 1 and 25 are ^{32}P-labelled λ DNA cut with *Hin*dIII and numbers are fragment sizes in kilo base pairs.



the sites for *Eco*RI, *Hae*II, and *Pvu*II. The nature of the *Hpa*I variation (i.e., whether it was due to a mutation at the restriction site or an insertion or deletion event) could not be discerned. There was an association between the *Hpa*I fragment patterns and those generated by *Hae*II ($P < 0.001$, $N = 30$), but

Table 3. Frequencies of different restriction fragment length polymorphisms (RFLPs) in *Pseudoterranova decipiens* from various hosts.

		Host species								
Restriction enzyme	RFLP	*Erignathus barbatus* (bearded seal)	*Halichoerus grypus* (grey seal)	*Phoca vitulina* (harbour seal)	*Gadus morhua* (Atlantic cod)	*Hemitripterus americanus* (sea raven)	*Hippoglossoides platessoides* (American plaice)	*Hippoglossus hippoglossus* (Atlantic halibut)	*Myoxocephalus scorpius* (shorthorn sculpin)	*Reinhardtius hippoglossoides* (turbot)
*Eco*RI	ADEF[a]	8	—	—	—	—	8	—	ns	2
	BCDEF	—	19	9	2	4	—	1	ns	1
	BDEF	—	3	—	—	—	—	—	ns	—
	CDEF	—	10	8	—	4	—	1	ns	—
*Hae*II	AB[a]	10	—	—	—	—	ns	ns	—	ns
	AC	—	2	1	—	—	ns	ns	—	ns
	ACD	—	9	16	3	3	ns	ns	3	ns
	AD	—	4	3	2	2	ns	ns	6	ns
*Hpa*I	AC	—	2	—	—	—	ns	ns	—	ns
	ACD	—	17	19	4	3	ns	ns	2	ns
	CD	—	3	3	1	2	ns	ns	6	ns
	BE	10	—	—	—	—	ns	ns	—	ns
*Pst*I	AC	6	—	—	ns	ns	ns	ns	ns	ns
	BC	—	9	10	ns	ns	ns	ns	ns	ns
*Pvu*II	AD	10	—	—	—	—	8	—	ns	2
	BCD	—	11	5	1	—	—	2	ns	1
	BD	—	2	1	—	—	—	—	ns	—
	CD	—	8	3	—	2	—	—	ns	—

Note: Genomic DNA was digested with the restriction enzyme and Southern blotted, and fragments were visualized by probing with a ^{32}P-labelled rDNA repeat (p*Dm*238). RFLPs were coded according to the fragment sizes listed in Table 2. Values are the number of specimens with the observed RFLP. ns, not screened.

[a]Fragment A in *Eco*RI digests and fragment B in *Hae*II digests appeared as 1–3 tightly spaced bands of variable intensity.

not with those produced by *Eco*RI ($P > 0.2$, $N = 16$) or *Pvu*II ($P > 0.5$, $N = 10$).

Intraindividual polymorphism

Comparison of the relative intensity of restriction fragments among lanes on the autoradiographs also indicated polymorphism in the number and (or) proportion of variant ribosomal repeat units within individuals, particularly within *P. decipiens* B. For example, differences are revealed by the *Hpa*I digests (Fig. 4) where some specimens (e.g., lane 17) have a preponderance of the 7.9-kbp fragment whereas in others (e.g., lane 18) the 5.5-kbp fragment is predominant. Similar differences are evident in Fig. 2 where the relative intensities of the 3.3- and 3.2-kbp *Eco*RI fragments differ considerably, indicating that the proportion of these variant repeats differs widely among individuals.

Geographic distribution and host specificity

RFLPs were surveyed in *P. decipiens* from seals and various species of fish collected widely across Atlantic Canada (Table 3). All 40 adult nematodes from bearded seals plus eight L3s from American plaice and two of three L3s from turbot showed RFLPs characteristic of *P. decipiens* C. Turbot was the only host found to harbour both *P. decipiens* B and *P. decipiens* C; one specimen from the Saguenay fjord (location 25, Fig. 1) was *P. decipiens* B, whereas both specimens from Labrador (location 4, Fig. 1) were *P. decipiens* C. The remaining specimens, which included 53 adult nematodes from grey seals, 46 adult nematodes from harbour seals, and 33 L3s from various species of fish, showed restriction fragment patterns characteristic of *P. decipiens* B. *Pseudoterranova decipiens* C was found only in waters off Labrador and northeast Newfoundland (localities 1–5, 8, 9, and 11, Fig. 1) whereas *P. decipiens* B was widely distributed from southern Labrador southward (localities 6, 7, 10, and 12–25, Fig. 1).

Discussion

Several studies have shown that comparison of RFLPs following hybridization with labelled rDNA probes is a powerful technique for revealing interspecific variation among closely related nematodes (e.g., Cameron et al. 1988; Boyd et al. 1989; Webster et al. 1990; Dame et al. 1991; Reid and Hominick 1992; Abu-Madi et al. 1994). In the present study the use of single digests and hybridization with a heterologous probe has proven particularly informative. Sequences in the coding regions of rDNA are highly conserved during evolution, whereas the spacer regions show little homology among species; consequently, the probe from *D. melanogaster* would be expected to hybridize only to fragments of nematode DNA that contain conserved sequences in the 18S and (or) 28S rRNAs. Other fragments generated by restriction sites within the variable spacer region would probably not be detected. In spite of this limitation this procedure revealed extensive rDNA restriction site variation within and among specimens of *P. decipiens* (sensu lato). Further studies involving double digests and other rDNA probes would be required to provide more detailed information on the numbers and positions of the restriction sites within the rDNA complex of *P. decipiens*.

Comparison of RFLPs indicates that there are two species within *P. decipiens* in Atlantic Canada. The definitive hosts and geographic distributions of each species are consistent with a previous study based on allozyme variation (Paggi et al. 1991). The allozyme study revealed that the two species showed fixed genetic differences at 7 of 16 enzyme loci. Our study reveals a total of 16 restriction fragments that could serve as additional genetic markers to distinguish *P. decipiens* B and *P. decipiens* C. We found no evidence that *P. decipiens* B in Atlantic Canada can be further subdivided into two variants as described by Appleton and Burt (1991). On the contrary, the presence of the same types and frequencies of rDNA polymorphisms in specimens from both grey and harbour seals strongly suggests that there is interbreeding between nematodes from these two species of seal.

The distribution of *P. decipiens* B and *P. decipiens* C in Atlantic Canada indicates a zone of sympatry off southern Labrador and northeast Newfoundland. Unfortunately, no samples from grey or harbour seals were available from this area to determine whether the two species were reproductively isolated by ecological factors or whether cross-infection of definitive hosts occurred. However, previously many larvae from this area were screened for diagnostic *Pgm* and (or) *Adk* alleles (see Paggi et al. 1991; Brattey and Stenson 1993 for methodology) and larvae of both species of *P. decipiens* were found in American plaice (75 of 76 specimens were *P. decipiens* C) and Atlantic cod (1 of 2 specimens was *P. decipiens* C) (J. Brattey, unpublished observations). Grey and harbour seals occur off southern Labrador (Boulva and McClaren 1979; Stobo et al. 1990) and both species feed on Atlantic cod and American plaice in Atlantic Canada (Benoit and Bowen 1990; Bowen et al. 1993); therefore, the co-occurrence of larvae of the two species of *P. decipiens* in seal prey suggests that opportunities for cross-infection and hence hybridization do occur, provided the nematodes develop to maturity. However, neither RFLP nor allozyme data give evidence of hybridization or introgression; therefore, *P. decipiens* B and *P. decipiens* C appear to be reproductively isolated.

Variation in rDNA sequences between and within individuals is known in several other taxa (Arnheim 1983), including rodents (Allard and Honeycutt 1991), amphibians (Wellauer et al. 1976), fish (Phillips et al. 1989), and insects (Long and Dawid 1980). However, there are few data on the nature and extent of intraspecific rDNA variation among individual parasitic nematodes (Dame et al. 1991; Anderson 1995; Hoste et al. 1995) because most nematodes are small, and several individuals must often be pooled or genetic material must be amplified by PCR to generate sufficient DNA for analysis. Our results indicate two types of rDNA repeat unit in *P. decipiens* B that differ by an insertion or deletion of 100 bp, as well as polymorphism in the number and (or) proportion of each repeat unit within individuals. This pattern of variation is common in multigene families and has been described in the evolution of rDNA (Dover and Coen 1981; Dover 1982). Interestingly, two types of repeat unit distinguished by an insertion or deletion have also been reported within the rDNA of individuals of *Ascaris* (Back et al. 1984).

Intraspecific polymorphism in rDNA sequences among *P. decipiens* B could be useful for investigating population subdivision across the broad geographic range of this species. Most of the polymorphisms we observed were found in specimens from several different hosts and localities across Atlantic Canada and our data give no indication of population subdivision

within this area. However, geographic coverage was too limited for a detailed population analysis. The allozyme study showed that *P. decipiens* B is distributed across a much broader area, including waters off Iceland, the United Kingdom, and Norway (Paggi et al. 1991), but too few polymorphic loci were found to investigate population subdivision in detail. Comparison of polymorphisms in the rDNA of *P. decipiens* B collected throughout the North Atlantic and adjacent areas could provide an alternative, more sensitive method of investigating population structuring within this species.

In conclusion, our data indicate extensive rDNA polymorphism among specimens of *P. decipiens* (sensu lato) and agree with a previous study of allozyme variation (Paggi et al. 1991) revealing two reproductively isolated species parasitizing fish and seals in Atlantic Canada. Bearded seals appear to be the only definitive hosts of *P. decipiens* C, whereas grey and harbour seals serve as definitive hosts for *P. decipiens* B. Larvae of *P. decipiens* C infect flatfishes off Labrador, whereas larvae of *P. decipiens* B are widely distributed and common in the flesh of several species of marine fish in Atlantic Canada. Similarities in both the types and frequencies of RFLPs in specimens from grey and harbour seals suggest that *P. decipiens* from these two species of seal are freely interbreeding.

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References

Abu-madi, M.A., Reid, A.P., Lewis, J.W., and Hominick, W.M. 1994. Genomic variability within laboratory and wild subspecies of *Heligmosomoides polygyrus*. J. Helminthol. **68**: 93–96.

Adamson, M.L., Clease, D.F., and Margolis, L. 1992. Differentiation of *Philonema* spp. (Nematoda: Philometridae) in British Columbia salmonid fishes using DNA restriction fragment length differences. Can. J. Fish. Aquat. Sci. **49**: 1650–1656.

Allard, M.W., and Honeycutt, R.L. 1991. Ribosomal DNA variation within and between species of rodents, with emphasis on the genus *Onchomys*. Mol. Biol. Evol. **8**: 71–84.

Anderson, T.J.C. 1995. Ascaris infections in humans from North America: molecular evidence for cross-infection. Parasitology, **110**: 215–219.

Appleton, T.E., and Burt, M.D.B. 1991. Biochemical characterization of third-stage larval sealworm, *Pseudoterranova decipiens* (Nematoda: Anisakidae), in Canadian Atlantic waters using isoelectric focusing of soluble proteins. Can. J. Fish. Aquat. Sci. **48**: 1800–1803.

Arnheim, N. 1983. Concerted evolution of multigene families. *In* Evolution of genes and proteins. *Edited by* M. Nei and R. K. Koehn. Sinaeur, Sunderland, Mass. pp. 38–61.

Back, E., Müller, F., and Tobler, H. 1984. Structural organization of the two main rDNA size classes of *Ascaris lumbricoides*. Nucleic Acids Res. **12**: 1313–1332.

Benoit, D., and Bowen, W.D. 1990. Seasonal and geographic variation in the diet of grey seal (*Halichoerus grypus*) in eastern Canada. *In* Population biology of sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and definitive hosts. *Edited by* W.D. Bowen. Can. Bull. Fish. Aquat. Sci. No. 222. pp. 215–226.

Boulva, J., and McLaren, I.A. 1979. Biology of the harbour seal, *Phoca vitulina*, in eastern Canada. Bull. Fish. Res. Board Can. No. 200.

Bowen, W.D. (*Editor*). 1990. Population biology of sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and definitive hosts. Can. Bull. Fish. Aquat. Sci. No. 222.

Bowen, W.D., Lawson, J.W., and Beck, B. 1993. Seasonal and geographic variation in the species composition and size of prey consumed by grey seals (*Halichoerus grypus*) on the Scotian Shelf. Can. J. Fish. Aquat. Sci. **50**: 1768–1778.

Boyd, D., deVos, T., Klassen, G., and Dick, T. 1989. Characterization of the ribosomal DNA from *Trichinella spiralis*. Mol. Biochem. Parasitol. **35**: 67–72.

Brattey, J., and Stenson, G.B. 1993. Host specificity and abundance of parasitic nematodes (Ascaridoidea) from the stomachs of five phocid species from Newfoundland and Labrador. Can. J. Zool. **71**: 2156–2166.

Brattey, J., Bishop, C.A., and Myers, R.A. 1990. Geographic distribution and abundance of *Pseudoterranova decipiens* (Nematoda: Acaridoidea) in the musculature of Atlantic cod, *Gadus morhua*, from Newfoundland and Labrador. *In* Population biology of sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and definitive hosts. *Edited by* W.D. Bowen. Can. Bull. Fish. Aquat. Sci. No. 222. pp. 67–82.

Cameron, M.L., Levy, P., Nutman, T., Vanamala, C.R., Narayanan, P.R., and Rajan, T.V. 1988. Use of restriction fragment length polymorphisms (RFLP's) to distinguish between nematodes of pathogenic significance. Parasitology, **96**: 381–390.

Curran, J., Baillie, D.L., and Webster, J.M. 1985. Use of genomic DNA restriction fragment length differences to identify nematode species. Parasitology, **90**: 137–144.

Dame, J.B., Yowell, C.A., Courtney, C.H., and Lindgren, W.G. 1991. Cloning and characterization of the ribosomal RNA gene repeat from *Ostertagia ostertagi*. Mol. Biochem. Parasitol. **45**: 275–280.

Dover, G.A. 1982. Molecular drive: a cohesive mode of species evolution. Nature (London), **299**: 111–117.

Dover, G.A., and Coen, E. 1981. Springcleaning ribosomal DNA: a model for multigene evolution. Nature (London), **290**: 731–732.

Grant, W.N., and Whitington, G.E. 1994. Extensive DNA polymorphism within and between two strains of *Trichostrongylus colubriformis*. Int. J. Parasitol. **24**: 719–725.

Hoste, H., Chilton, N.B., Gasser, R.B., and Beveridge, I. 1995. Differences in the second internal transcribed spacer (ribosomal DNA) between five species of *Trichostrongylus* (Nematoda: Trichostrongylidae). Int. J. Parasitol. **25**: 75–80.

Karl, S.A., and Avise, J.C. 1992. Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science (Washington, D.C.), **256**: 100–103.

Klassen, G.R., Thiessen, J.P., and Dick, T.A. 1986. Strain-specific 1.7 kilobase repetitive deoxyribonucleic acid sequence family in *Trichinella spiralis*. Mol. Biochem. Parasitol. **21**: 227–233.

Long, E.O., and Dawid, I.B. 1980. Repeated genes in eukaryotes. Annu. Rev. Biochem. **49**: 727–764.

Malouf, A.H. (*Chairman*). 1986. Report of the Royal Commission on seals and sealing in Canada. Vol. 3. Canadian Government Publishing Centre, Ottawa, Ont.

Maniatis, T., Fritsch, E.F., and Sambrook, J. 1982. Molecular cloning. A laboratory manual. Cold Spring Harbour Laboratory, Cold Spring Harbor, N.Y.

Margolis, L. 1977. Public health aspects of "codworm" infection: a review. J. Fish. Res. Board Can. **34**: 887–898.

Matsuura, T., Sun, T.S., and Sugane, K. 1992. The identity of *Anisakis* type II larvae with *Anisakis physeteris* confirmed by restriction fragment length polymorphism analysis of genomic DNA. J. Helminthol. **66**: 33–37.

McClelland, G., Misra, R.K., and Marcogliese, D.J. 1983*a*. Variations in abundance of larval anisakines, "sealworm" (*Phocanema decipiens*) and related species, in cod and flatfish from the southern Gulf of St. Lawrence (4T) and the Breton Shelf (4Vn). Can. Tech. Rep. Fish. Aquat. Sci. No. 1201.

McClelland, G., Misra, R.K., and Marcogliese, D.J. 1983*b*. Variations in abundance of larval anisakines, "sealworm" (*Phocanema decipiens*) and related species, in Scotian Shelf (4Vs and 4W) cod and flatfish. Can. Tech. Rep. Fish. Aquat. Sci. No. 1202.

McClelland, G., Misra, R.K., and Martell, D.J. 1985. Variations in abundance of larval anisakines, sealworm (*Pseudoterranova decipiens*) and related species, in eastern Canadian cod and flatfish. Can. Tech. Rep. Fish. Aquat. Sci. No. 1392.

McClelland, G., Misra, R.K., and Martell, D.J. 1990. Larval anisakine nematodes in various fish species from Sable Island Bank and vicinity. *In* Population biology of sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and seal hosts. *Edited by* W.D. Bowen. Can. Bull. Fish. Aquat. Sci. No. 222. pp. 83–118.

Nagano, K. 1989. Gastric terranoviasis. *In* Gastric anisakiasis in Japan. *Edited by* H. Ishikura and M. Namiki. Springer-Verlag, Tokyo, Japan. pp. 133–140.

Paggi, L., Nascetti, G., Cianchi, R., Orecchia, P., Mattiucci, S., D'Amelio, S., Berland, B., Brattey, J., Smith, J.W., and Bullini, L. 1991. Genetic evidence for three species within *Pseudoterranova decipiens* (Nematoda, Ascaridida, Ascaridoidea) in the North Atlantic and Norwegian and Barents Seas. Int. J. Parasitol. **21**: 195–212.

Philips, R.B., Pleyte, K.A., van Ert, L.M., and Hartley, S.E. 1989. Evolution of nucleolar organizer regions and ribosomal RNA genes in *Salvelinus*. Physiol. Ecol. Jpn. Spec. Vol. 1. pp. 429–447.

Reid, A.P., and Hominick, W.M. 1992. Restriction fragment length polymorphisms within the ribosomal DNA repeat unit of British entomopathogenic nematodes (Rhabditida: Steinernematidae). Parasitology, **105**: 317–323.

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**: 503–517.

Stobo, W.T., Beck, B., and Horne, J.K. 1990. Seasonal movements of grey seals (*Halichoerus grypus*) in the Northwest Atlantic. *In* Population biology of sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and definitive hosts. *Edited by* W.D. Bowen. Can. Bull. Fish. Aquat. Sci. No. 222.

Sugane, K., Qing, L., and Matsuura, T. 1989. Restriction fragment length polymorphisms of Anisakinae larvae. J. Helminthol. **63**: 269–274.

Templeman, W., Squires, H.J., and Fleming, A.M. 1957. Nematodes in the fillets of cod and other fishes in Newfoundland and neighbouring areas. J. Fish. Res. Board Can. **14**: 831–897.

Webster, J.M., Anderson, R.V., Baillie, D.L., Beckenbach, K., Curran, J., and Rutherford, T. 1990. DNA probes for differentiating isolates of the pinewood nematode species complex. Rev. Nematol. **13**: 255–263.

Wellauer, P.K., Dawid, I.B., Brown, D.D., and Reeder, R. 1976. The molecular basis for length heterogeneity in ribosomal DNA from *Xenopus*. J. Mol. Biol. **105**: 461–486.

DNA fingerprinting of bluegill sunfish (*Lepomis macrochirus*) using $(GT)_n$ microsatellites and its potential for assessment of mating success

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Abstract: Variable microsatellite DNA markers are powerful tools to study parentage and gene flow in fish populations. In this work, we cloned and characterized 60 (GT) repeat microsatellite sequences from the bluegill sunfish (*Lepomis macrochirus*) genome. We report the frequency distribution, the lengths, and the proportions of the repeat classes for comparison with microsatellites isolated from other teleost, mammal, and invertebrate genomes. Bluegill microsatellites are similar to those of other fish, strengthening previous reports of significant differences in the organization of microsatellites between teleosts and mammals. Primers for polymerase chain reaction amplification were designed for 17 bluegill microsatellite loci. The allele frequencies, the degree of polymorphism, and heterozygosities were estimated using bluegill from Lake Opinicon, Ontario. Seven loci are potentially highly informative for fingerprinting studies of parentage. A preliminary paternity analysis using two of these loci on brood from a natural nest revealed their potential utility at estimating relative success of the two reproductive life histories, parental and cuckolder, characteristic of male bluegill. Cross-amplification using these microsatellite markers on related fish species showed some degree of locus conservation among taxa, potentially allowing DNA fingerprinting of a variety of centrarchids.

Résumé : Les marqueurs à ADN microsatellite variable sont des outils puissants pour l'étude de la filiation et des mouvements génétiques dans les populations de poissons. Au cours de cette étude, nous avons cloné et caractérisé 60 séquences d'ADN microsatellite répétitives (GT) provenant du génome du crapet arlequin (*Lepomis macrochirus*). Nous indiquons la fréquence, la distribution, les longueurs et les proportions des classes répétitives pour des comparaisons avec des microsatellites isolés du génome d'autres téléostéens, mammifères et invertébrés. Les microsatellites du crapet arlequin sont semblables à ceux d'autres poissons, ce qui semble confirmer des rapports antérieurs faisant état de différences significatives entre les téléostéens et les mammifères dans l'organisation des microsatellites. On a conçu des amorces pour l'amplification de la réaction en chaîne de la polymérase à 17 locus d'ADN microsatellites du crapet arlequin. À l'aide de crapets arlequins du lac Opinicon (Ontario), on a estimé la fréquence des allèles, le degré de polymorphisme et les hétérozygosités. Sept de ces locus pourraient être des sources importantes d'information pour les études de la filiation grâce à l'identification par le code génétique. Une analyse préliminaire de paternité utilisant deux de ces locus avec les petits d'un nid naturel montre leur utilité possible pour l'estimation du succès relatif des deux stratégies de reproduction, par les couples et hors couple, caractéristiques du crapet arlequin mâle. L'utilisation de l'amplification croisée avec ces marqueurs à ADN microsatellite pour des espèces de poissons apparentées indique qu'il y a un certain degré de conservation des locus d'un taxon à l'autre, ce qui rend possible l'identification par le code génétique chez divers centrarchidés.
[Traduit par la Rédaction]

Introduction

Polymorphic DNA markers can provide fisheries researchers with new insight into the behaviour, ecology, and genetic structure of fish populations (Wirgin and Waldman 1994). Genetic markers capable of tracking the contribution of individual parents to the recruitment generation can yield valuable information regarding effective population sizes (Withler and Beacham 1994), levels of inbreeding (Gross et al. 1994), disassortive mating, the success of alternative reproductive strategies and life histories (Hutchings and Myers 1988; Rico et al. 1992; Philipp and Gross 1994), and the intensity of natural and sexual selection (Schroder 1981; Fleming and Gross 1994). Furthermore, gene flow studies using neutral DNA markers are relevant to the conservation and management of fish stocks (Hedrick 1994; Wirgin and Waldman 1994). However, the majority of genetic markers that are available to researchers are not ideally suited for investigations of parentage and kinship. Allozymes and restriction fragment length polymorphisms (RFLPs) can be too limited in allelic variation to

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estimate relatedness along recent lineages (Queller et al. 1993). Randomly amplified polymorphic DNA (RAPDs) may be problematic when comparing banding patterns from different gels, and researchers using multilocus fingerprinting may incur high costs (Lewin 1989; Weatherhead and Montgomerie 1991). For these reasons, a powerful class of highly polymorphic genetic markers called microsatellites is increasingly being used in parentage and identity testing (Queller et al. 1993; Wright and Bentzen 1994; O'Reilly and Wright 1995).

Microsatellites are tandemly arrayed noncoding sequences consisting of very short nucleotide motifs and their lengths are usually no larger than 300 base pairs (bp) (Tautz 1989; Wright 1993). Generally, they are abundant and distributed throughout the eukaryotic genome (Tautz and Renz 1984). Within vertebrates, the dinucleotide repeat $(GT)_n$ is believed to be the most common microsatellite (Brenner et al. 1993). DNA fingerprinting using these sequences makes use of the polymerase chain reaction (PCR), whereby tandemly repeated polymorphic segments of minute DNA samples are amplified prior to resolving their lengths. This amplification presents a significant advantage over other non-PCR based methods because it allows the use of relatively small amounts of tissue, including that from preserved otoliths, scales, larvae, and small fry. Allelic variants of a microsatellite locus are codominant and show Mendelian inheritance. Moreover, because microsatellite loci often have many alleles and show high heterozygosity, parentage may be ascertained unambiguously using as few as 6–10 microsatellite markers.

An ongoing research interest of one of our laboratories has been the evolution of alternative reproductive life histories such as that shown by the behaviour of bluegill sunfish (*Lepomis macrochirus*). Bluegill sunfish are found in warm freshwater lakes throughout eastern and central North America (Scott and Crossman 1973). In Lake Opinicon (eastern Ontario), these iteroparous fish spawn from May through July in colonies of about 60 nests (Cargnelli and Gross 1996). The bluegill mating system is characterized by a discrete polymorphism in male behaviour and life history (Gross 1982). Parental males first mature at age 7 or 8 years, then compete for nesting territory in colonies prior to spawning. After spawning, these males remain at their nests for 5–12 days to care for and guard their brood. Other males, called cuckolders, mature precociously at age 2 and successfully steal fertilizations in the nest of parental males (Philipp and Gross 1994), never becoming parental males themselves (Gross 1982). Cuckolders thus leave the care of their offspring to the parental males. Similar alternative life histories are found in many fish species (Gross 1984). The evolution of such alternative reproductive life histories has been hypothesized to be explained in part by negative frequency-dependent sexual selection, whereby the mating success of a life history is inversely related to its frequency in the population (Gross 1985, 1991). Genetic markers are necessary to test this hypothesis by measuring the relative fitness of the alternative life histories. Such genetic markers for bluegill have not previously been available.

This study (*i*) describes the cloning and characterization of microsatellites from bluegill, (*ii*) compares the structure and genomic organization of $(GT)_n$ microsatellites from bluegill to microsatellites isolated from other taxa, and (*iii*) demonstrates the utility of the microsatellites for quantifying fitness by measuring the paternity within a wild parental bluegill's nest.

Materials and methods

Cloning and sequencing of microsatellite loci

A partial genomic library of bluegill sunfish DNA was constructed for the screening of microsatellites. High molecular weight DNA from 0.6 g of liver tissue of a Lake Opinicon bluegill was extracted using the method described in Coen et al. (1982) with few modifications. One hundred micrograms of genomic DNA was digested to completion with restriction endonucleases *Hae*III, *Hin*cII, and *Rsa*I (Pharmacia) according to the manufacturer's instructions. The digestion product was size fractionated by agarose gel electrophoresis and the DNA fragments between 300 and 800 nucleotides in length were excised from the gel. This range of fragment sizes was chosen because DNA fragments of these lengths are short enough to be easily sequenced, yet relatively large enough to provide adequate sequence information up- and down-stream from an average-sized microsatellite region. After electroelution, the DNA was resuspended in 1× TE buffer (10 mM Tris-HCl (pH 8.0); 0.1 mM ethylenediaminetetraacetic acid (EDTA)).

Using excess T4 DNA ligase (Pharmacia), the size-selected DNA fragments were blunt-end ligated into 20 ng of dephosphorylated pUC18 vector cut at the *Sma*I cloning site. Reactions (25 μL) were conducted at three different vector:insert molar ratios (1:0.5, 1:1, and 1:2). After the transformations of DH5α competent cells (Gibco BRL) with 2 μL of the ligation reaction, the plasmids were multiplied according to standard protocols (Sambrook et al. 1989).

Recombinant clones were cultured on solid Luria-Bertani (LB) medium plates containing 100 μg/mL ampicillin. After the size-selected library was transferred onto Hybond-N nylon membranes (Amersham), the cloned DNA was immobilized onto membranes by baking at 80°C, and then screened for microsatellites by hybridization with a synthetic $(GT)_{15}$ oligonucleotide. The oligonucleotide was 5′ end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (Pharmacia). The filters were hybridized to the oligonucleotide probe at 58°C in 5× SSPE (0.9 M NaCl; 50 mM NaH_2PO_4; 5 mM EDTA); 5× Denhardt's (0.5% ficoll 400; 0.5% polyvinylpyrolidone; 0.5% bovine serum albumin); 0.5% sodium dodecyl sulphate (SDS); and 100 μg/mL RNA after a 9-h prehybridization in the same solution. The membranes were washed once with 2× standard saline citrate (SSC) – 0.2% SDS at room temperature for 15 min and once with 0.2× SSC – 0.2% SDS at 60°C for 5 min (see Sambrook et al. 1989). Clones having DNA inserts containing $(GT)_n$ microsatellites were identified by exposing the membranes to Kodak X-Omat AR film at –70°C with intensifying screens. Positive recombinants were picked from the plates and grown in LB liquid medium. Plasmid DNA was then purified (Goode and Feinstein 1992), alkaline denatured, and sequenced by the dideoxynucleotide chain termination method (Sanger et al. 1977) using T7 DNA polymerase (Pharmacia).

PCR primers (forward and reverse primers) were designed and synthesized complementary to unique sequences flanking 19 microsatellite loci. These microsatellites and the site of primer annealing were chosen to give PCR products in the range of 80–200 bp, a size readily resolved on DNA sequencing gels.

PCR amplification

PCR conditions were optimized for the annealing of each set of primers to the template DNA by systematically increasing or decreasing the temperature. An initial annealing temperature (T_{ann}) of 50°C was chosen. Polymerase chain reactions were conducted in volumes of 10 μL, overlaid with light mineral oil. Standard reaction mixtures contained 10–20 ng of template DNA, 0.6 μM of the forward primer, 0.56 μM of the reverse primer, 200 μM each of dATP, dCTP, dGTP, and dTTP, 1× reaction buffer (10 mM Tris-HCl (pH 8.3), 1 mM $MgCl_2$, and 50 mM KCl), 0.25 units of *Taq* polymerase (Perkin Elmer), and 0.04 μM of reverse primer 5′ end-labeled with [γ-^{32}P]ATP (37 kBq/10 pmol primer) using T4 polynucleotide kinase (Pharmacia). Amplifications were performed using a Perkin Elmer 480 thermal

Table 1. Density of $(GT)_n$ microsatellites in the genomes of various fish, mammal, and invertebrate species, compared with that of bluegill.

Species	Dispersion (kbp^{-1})	Relative dispersion[a]	Source
Fish			
Bluegill (*Lepomis macrochirus*)	14	1.0	The present study
Atlantic cod (*Gadus morhua*)	7	0.5	Brooker et al. 1994
Atlantic salmon (*Salmo salar*)	90	6.4	Slettan et al. 1993
Brown trout (*Salmo trutta*)	23	1.6	Estoup et al. 1993*a*
Zebrafish (*Percina caprodes*)	12	0.9	Goff et al. 1992
Mammals			
Human (*Homo sapiens*)	28	2.0	Stallings et al. 1991; Beckmann and Weber 1992
Mouse (*Mus* sp.)	18	1.3	Stallings et al. 1991
Rat (*Rattus* sp.)	21	1.5	Stallings et al. 1991
Porcine (*Sus* sp.)	46	3.3	Wintero et al. 1992
Invertebrates			
Honeybee (*Apis mellifera*)	34	2.4	Estoup et al. 1993*b*
Snail (*Buliinus truncatus*)	40–45	3	Jarne et al. 1994

[a](Microsatellite dispersion (kbp^{-1}))/(bluegill microsatellite dispersion (kbp^{-1})).

cycler. After a 1-min denaturation at 94°C, samples were subjected to 7 cycles consisting of 1 min at 94°C, 1.0 min at the optimal T_{ann} and 30 s at 72°C, followed by 23 cycles of 45 s at 92°C, 1.0 min at T_{ann}, and 30 s at 72°C. One part deionized formamide stop dye was added to each reaction vessel followed by denaturation at 95°C for 10 min. Two microlitres of each sample was loaded onto a 6% polyacrylamide urea gel and subjected to electrophoresis in 1× TBE buffer (89 mM Tris-borate (pH 8.0); 20 mM EDTA) alongside an M13mp18 sequence to size the PCR products. Once dried, the gel was exposed overnight at room temperature to X-ray film. If adequate PCR product was not obtained by varying the annealing temperature, the $MgCl_2$ concentration was titrated from 2 to 0.5 mM in the reaction buffer to vary the annealing stringency of the primers to the DNA template.

Paternity application

We tested the utility of 19 microsatellite loci for use in paternity analysis. First, to demonstrate polymorphism and establish allele frequencies for each locus, from 5 to 174 unrelated bluegill were fingerprinted. Second, to demonstrate Mendelian inheritance of the alleles, three males were crossed with three females in containment facilities at the Queen's University Biological Station, Lake Opinicon, and their fry fingerprinted. Third, to demonstrate the utility of microsatellites for field samples, a tissue sample was collected from a nesting parental male bluegill and whole fry were taken from his nest by skin divers after observing cuckoldry (in June 1993). The field samples were preserved in 90% ethanol and later fingerprinted to calculate cuckolder success.

Results and discussion

Density of $(GT)_n$ microsatellites in the bluegill genome

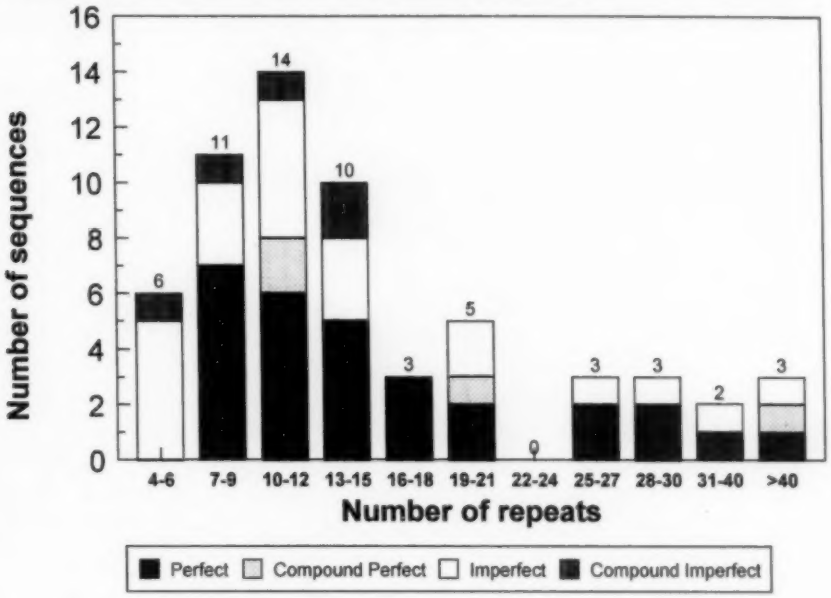
A total of 70 microsatellites were isolated and sequenced from a partial genomic library composed of 2035 clones. Of these 70 microsatellites, 60 were (GT) repeats. The average size of the clonal inserts was about 400 bp. Therefore, the total number of base pairs that were analyzed is estimated to be $400 \times 2035 = 8.14 \times 10^5$ bp. Assuming that the partial genomic library was representative of the bluegill genome, and that the $(GT)_n$ sequences are evenly distributed throughout the genome, a crude estimate of the average distance between neighbouring microsatellites is 14 kilo base pairs (kbp) ($(8.14 \times 10^5$ bp)/60). On the basis of this estimate, it appears that bluegill $(GT)_n$ microsatellites are more abundant than those reported in mouse, rat, human, honeybee, snail, and pig genomes (Table 1). When compared with other fish, bluegill repeats are about as abundant as those of zebrafish and more abundant than those in brown trout and Atlantic salmon. However, they are less abundant than those in Atlantic cod. Because of the varying stringencies of hybridizations used by investigators during screening of genomic libraries for microsatellites, these comparisons must be interpreted with caution. Nonetheless, the sunfish genome has a relatively large number of $(GT)_n$ microsatellites.

Length and sequence of bluegill microsatellites

The cloned microsatellites were partitioned into three categories, perfect, imperfect, and compound as defined by Weber (1990), giving the results presented in Fig. 1 and Table 2. Perfect microsatellites are uninterrupted series of a repeat unit. Imperfect microsatellite sequences differ from perfect ones by the presence of no more than three bases that arrest an alternating tandem repeat, such that its terminal ends on either side are at least three full repeats in length. Compound repeat sequences consist of several different repeat types and are separated by less than three intervening bases. Compound repeats are further subdivided as either perfect or imperfect sequences, depending upon the condition of the individual microsatellite units.

The predominant category of bluegill $(GT)_n$ microsatellites are the perfect repeats, while the most common size-class including all categories contains sequences with lengths of 10–12 repeats. Imperfect and perfect microsatellites have approximately the same size distribution, regardless of whether one measures the longest run of uninterrupted repeats, or the overall number of repeats within their sequences. Only one compound microsatellite locus had more than two different repeated motifs (two of these motifs had over 40 repeats). In all, this microsatellite comprised over 90 dinucleotides. Although only 10 compound repeats were sequenced, a wide variety of associated microsatellites were observed. Of these, $(GT)_n$ microsatellites were found with neighbouring tandem

Fig. 1. Frequency of different size-classes measuring the longest uninterrupted microsatellite arrays from bluegill sunfish. Size-classes are labeled as number of repeat units.



repeats of one to six nucleotides. This variety was also noted in the honeybee (Estoup et al. 1993*b*).

Brooker et al. (1994) found that representative teleost microsatellites are significantly larger and show greater degeneracy in their tandem repeats than do mammalian microsatellites. They suggested that this difference in repeat sequences may be due to more frequent slippage events during DNA replication in a cold-water environment. The bluegill microsatellites share many similarities with the three fish species, Atlantic cod (*Gadus morhua*), brown trout (*Salmo trutta*), and Atlantic salmon (*Salmo salar*), studied by Brooker et al. (1994). First, the proportions of bluegill microsatellites falling within Weber's (1990) three categories closely resemble those of Atlantic cod and brown trout. Among these fish, almost 50% of the microsatellites are categorized as being imperfect and thus degenerate in repeat sequence. Second, bluegill and the other three fish species share their most frequent microsatellite size-class (which is moderately shorter than the most frequent size-class in mammals). Third, the microsatellites of all four species are on average longer than those of mammals. Furthermore, the fish microsatellites can be quite large, while those of mammals rarely exceed 30 repeats. Fourth, all four fish species tend toward a bimodal distribution of microsatellite size-classes (although in bluegill the second peak is not as dominant).

Recently, dinucleotide microsatellite repeats have also been identified in invertebrates. Like fish, some of the $(GT)_n$ microsatellites in a snail (*Buliinus truncatus*) are large, exceeding 50 repeats (Jarne et al. 1994). Honeybees (*Apis mellifera*; Estoup et al. 1993*b*) have the same proportions of microsatellites in perfect and imperfect categories as do the fish, and some exceed 85 repeats in length. Therefore, the difference in size and degeneracy of microsatellites between fish and mammals may be a characteristic not restricted to cold-water environments, but shared by many poikilotherms.

Table 2. Number of microsatellite sequences in different repeat categories (perfect, imperfect, and compound; see text) in bluegill.

Category	$(GT)_n$/ $(AC)_n$	$(CT)_n$/$(GA)_n$ and $(AT)_n$	Polynucleotides	Total No.	Total %
Perfect	29	2	2	33	47
Imperfect	22	2	3	27	39
Compound (perfect)	4	1	0	5	7
Compound (imperfect)	5	0	0	5	7

Note: Perfect dinucleotide microsatellites have ≥6 motifs, and the longest runs of dinucleotide repeats in close association with other microsatellites have ≥4.

Variability of bluegill microsatellites

PCR primers were designed for 19 loci. Of these primer sets, 17 provided scorable product. When the primer sets were used to amplify loci from unrelated individuals, 7 loci were found to be polymorphic with 2–8 alleles. The heterozygosities of these loci range from 27 to 63% (Table 3).

Many microsatellites (71%), although large, are not polymorphic. Weber (1990) noted that the degree of microsatellite polymorphism in humans is positively correlated with the size of the microsatellite array. However, bluegill microsatellites vary in the extent of their polymorphisms, apparently independently of size. Even sequences that are of the same category and size-class as other polymorphic loci may or may not exhibit polymorphism. This finding suggests that factors other

Table 3. Nucleotide and primer sequences, optimal annealing temperature, and $MgCl_2$ concentration for PCR amplification of the 17 bluegill sunfish microsatellite loci.

Locus	Locus sequence	Primer sequences	*N*	No. alleles	Size (bp)	% H	T_{ann} (°C)	$[MgCl_2]$ (mM)
Lma 10	$(TG)_{10}(TATGTG)_4$	5′ GTCTGTAAGTGTGTTTGCTG	57	4	117–134	56	50	1.5
		5′ GAAACCCGAAACTTGTCTAG						
Lma 12	$(ACACAT)_2(ACACAC)(ACACAT)_4$	5′ CTGCTCAGCATGGAGGCAG	171	8	89–135	27	58	1.5
		5′ TTCTTCCACAATATTCTCGCG						
Lma 20	$(AC)_4N(CA)_{25}$	5′ GGCACTAATCTAATTGTAGCC	155	8	85–117	62	64	1.0
		5′ TTGTGTGTCTGCATTGGAATC						
Lma 21	$(TC)_{19}(AC)_{11}$	5′ CAGCTCAATAGTTCTGTCAGG	174	6	154–182	60	58	0.5
		5′ ACTACTGCTGAAGATATTGTAG						
Lma 25	$(GT)_{18}$	5′ CATTGGAAGTGGCTGCAAGC	36	1	129	0	50	1.5
		5′ CAGACAGGTCAGACCTGCTC						
Lma 26	$(TG)_{12}$	5′ TAATATACCAAACCTGCATGTC	15	1	124	0	54	1.0
		5′ CTCACAGGGAATAAACCGTC						
Lma 29	$(GT)_{30}$	5′ CCCTGTTACTTGTGTATTC	138	5	85–118	63	54	1.0
		5′ ATTCAGAGGCAAGCATTATC						
Lma 35	$(TG)_7N(GT)_{20}$	5′ CAGCCCCATGTATGAAGAC	15	2[a]	113, 145	—	50	1.5
		5′ CCTACACCAATAGGAGGTGC						
Lma 44	$(CA)_{10}N(AC)_4(ACC)_3$	5′ CAGTGATTTCCATTCCTCATC	15	2	98, 102	28	50	1.5
		5′ CTGCTGCCAGAGACAATGAC						
Lma 45	$(AAG)_4N_{11}(GT)_{17}$	5′ CACGAACCACTGAAGCTCAG	5	1	106	0	50	1.5
		5′ ATAGCAGGTCGGGTTCACTG						
Lma 54	$(TG)_{30}$	5′ CGCCTCGCCATGCTCAGTG	10	1	132	0	57	1.0
		5′ AGTGTTTCAGGGACAGTAACAG						
Lma 55	$(TG)_{13}NN(TG)_6N_6(GT)_5(GCGT)_3$	5′ TCAACATGCCAGCACTCAG	5	1	123	0	50	1.5
		5′ CTGTATTTTGACTCTAGAGG						
Lma 57	$(CA)_{20}NN(CA)_3$	5′ AAGATGTGGATGGCCTCAAC	5	1	102	0	50	1.5
		5′ ACATGGCAGAGGGTTGTGAC						
Lma 71	$(TG)_9$	5′ TGCGGGCAGCTCGGTCTGG	30	2[a]	146, 167	—	50	1.5
		5′ TCTCTCTGCCTGCATGTAGCTG						
Lma 78	$(TCA)_5NNN(TCA)_5$	5′ TAATGCTCTGACTGAAGGAG	20	2[a]	113, 122	—	50	1.5
		5′ CAGTTAAAGGCATGGCGAAG						
Lma 86	$(CA)_{39}(GA)_n(TG)_n$	5′ CAGAGTTACTGCAATCAGCTC	10	1	240	0	50	1.5
		5′ CAGATGCAGGTGTGCTACTC						
Lma 87	$(AC)_{15}(A)_5$	5′ ATGACACAGACTCACCATGC	172	4	118–151	53	58	0.5
		5′ CTCCTGCCCATAAATCAGAC						

Note: Number of alleles, sizes of PCR products, and percent heterozygosity (calculated from allele frequencies) were determined from *N* unrelated individuals collected from Lake Opinicon, Ontario.

[a] Primers amplify two microsatellite loci that are each fixed for one allele.

than length may be responsible for allelic variation at microsatellite loci (see also Wright 1994).

Paternity application to bluegill sunfish

Several of the microsatellites isolated from bluegill were evaluated for diagnosing paternity in Lake Opinicon bluegill sunfish. The test crosses revealed Mendelian inheritance of alleles for the five polymorphic loci that were tested. Each fry from the test crosses had an expected allele from each parent (Table 4).

The polymorphic microsatellite loci, Lma 21 and Lma 87, were amplified from a parental male and 10 randomly selected fry from a natural nest where cuckoldry had been observed (Fig. 2).

The parental male's genotype at loci Lma 21 and Lma 87 was 154/160 and 128/128, respectively. All progeny sired by this male should inherit one of his alleles at each of these loci. Therefore, his progeny should have either the 154 or the 160 allele at locus Lma 21 and the 128 allele at locus Lma 87. Of the 10 fry, 3 can be excluded as progeny of the parental male. These are individual 3 (genotype 164/164 and 118/118 at loci Lma 21 and Lma 87, respectively) and individual 6 (164/164 and 118/133), which do not share an allele with the parental male at either locus, and individual 8 (160/164 and 118/133), which shares an allele with the parental male at locus Lma 21 but does not share an allele with the parental male at locus Lma 87.

Examining the genotypes for the fry at Lma 87 in Fig. 2, and assuming that in addition to the parental male (128/128) there were only one female and one cuckolder male contributing to the fry, it is possible to predict the genotypes of that female and cuckolder. Fry 1 is 128/128 and therefore received the 128 allele from both the mother and father. Fry 3 is 118/118 and by a similar argument the mother must possess

Table 4. Inheritance data of five polymorphic bluegill microsatellite loci tested with full-sib families.

Full-sib family	Locus	Female	Male	Offspring					
				AA	AA′	A′A′	AA″	A′A″	A″A″
1	Lma 10	119/128 (AA′)	128/128 (A′A′)	—	20	18	—	—	—
1	Lma 12	128/128 (AA)	128/128 (AA)	35	—	—	—	—	—
2	Lma 10	117/119 (AA′)	128/128 (A″A″)	—	—	—	10	16	—
2	Lma 44	102/102 (AA)	102/102 (AA)	31	—	—	—	—	—
3	Lma 21	160/164 (AA′)	164/164 (A′A′)	—	12	10	—	—	—
3	Lma 87	128/128 (A′A′)	118/118 (AA)	—	21	—	—	—	—

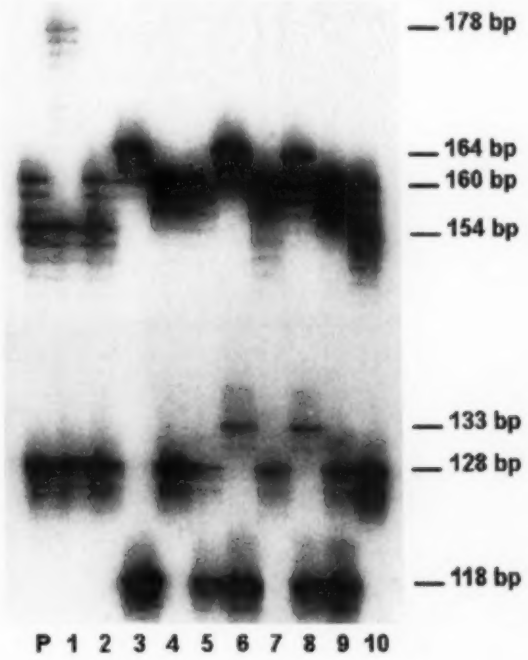
the 118 allele. Having assessed the mother's genotype as 118/128, it is possible to determine the genotype of the assumed single cuckolder male. Since fry 3 was identified previously as unrelated to the parental male, the cuckolder male must be the father and possess an 118 allele. Fry 6 and fry 8 were also identified previously as unrelated to the parental male, and since the mother does not possess the 133 allele, the 133 allele in their 118/133 genotype must come from the cuckolder. The cuckolder male's genotype is therefore 118/133.

The assumption of only one mother is not sustained when considering the second locus. Examining the genotypes for the fry at Lma 21 in Fig. 2, it is possible to determine that there was an additional female contributing to the fry in this brood. Fry 3 and fry 4 are homozygous for the 164 and 160 alleles, respectively. If there was only one mother she must possess both of these alleles (160/164). But fry 1 (154/178) does not possess either the 160 or 164 allele. Thus, at least three maternal alleles exist (160, 164, and either 154 or 178) and therefore there are at least two mothers. Because fry 3 (164/164) has already been identified as fathered by a cuckolder male, allele 164 is also a cuckolder's allele. Finally, if the 154 allele is maternal then the 178 allele from fry 1 must also be a cuckolder's as the parental male (154/160) does not possess this allele.

Utilizing both loci we have determined that (*i*) in addition to the parental male there were at least one cuckolder and at least two females contributing to the brood; (*ii*) the mothers must possess the 118 and 128 alleles at Lma 87, and the 160, 164, and either 154 or 178 alleles at Lma 21; (*iii*) the cuckolder(s) must possess the 118 and 164 alleles; and (*iv*) the 178 allele also belongs to the cuckolder(s) if the 154 allele is maternal. The ability to detect progeny not sired by a putative parent, and to determine the number and genotypes of the unknown parents, increases with the number of polymorphic microsatellite loci utilized.

In rare circumstances, a fry may be falsely assigned as a cuckolder's offspring for having none of the paternal parent's alleles at a single locus because of the relatively high rates of mutation at microsatellite loci. Dallas (1992) estimated microsatellite mutation rates at 10^{-4} to 10^{-3}.

Fig. 2. PCR-amplified microsatellite alleles of a parental male (P) and 10 randomly selected fry from a Lake Opinicon bluegill nest using Lma 21 (alleles 154–178 bp) and Lma 87 (alleles 118–133 bp). The genotypes at loci Lma 21 and Lma 87 are as follows: (P) 154/160, 128/128; fry (1) 154/178, 128/128, (2) 154/160, 128/128, (3) 164/164, 118/118, (4) 160/160, 128/128, (5) 160/160, 118/128, (6) 164/164, 118/133, (7) 154/160, 128/128, (8) 160/164, 118/133, (9) 160/160, 118/128, and (10) 154/160, 128/128. Fry numbers 3, 6, and 8 do not share alleles with the parental male at one or both loci; thus, they were sired by a cuckolder. Sizes of alleles were estimated using an M13mp18 sequence ladder (not shown).



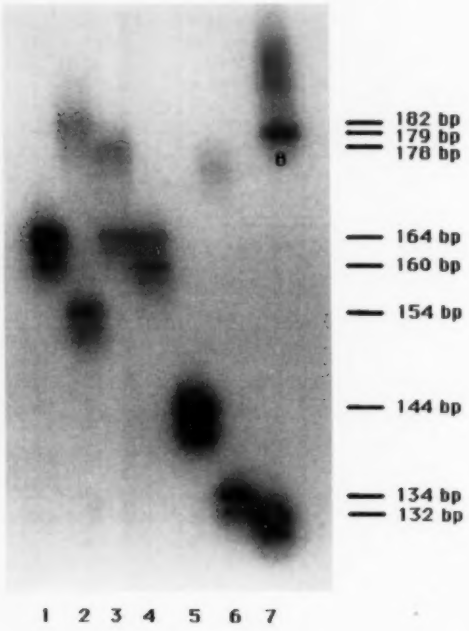
Cross-amplifications using bluegill primers

Selected primer sets that we developed for bluegill were tested on four other species. Primer sets for bluegill microsatellite loci Lma 20, Lma 21, Lma 29, and Lma 87 amplified DNA from pumpkinseed sunfish (*Lepomis gibbosus*); Lma 21 and 87 primers amplified DNA from largemouth bass (*Micropterus salmoides*) and rock bass (*Ambloplites rupestris*), and Lma 21 primers amplified DNA from yellow perch (*Perca flavescens*; Fig. 3).

For all four sets of primers, no changes to the PCR conditions were required. Thus, there has been evolutionary conservation of these loci, which may indicate their utility in phylogenetic and broader paternity studies.

In summary, the microsatellites presented here provide a useful comparison with those of other organisms, and also a

Fig. 3. PCR products of various alleles at the Lma 2.1 locus from bluegill (1–4), a pumpkinseed (5), a largemouth bass (6), a rockbass (7), and a perch (8). Sizes of the PCR products were estimated using an M13mp18 sequence ladder (not shown).



potential tool for parentage analyses of fish mating systems in nature.

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References

Beckmann, J.S., and Weber, J.L. 1992. Survey of human and rat microsatellites. Genomics, **12**: 627–631.

Brenner, S., Elgar, G., Sandford, R., Macrae, A., Venkatesh, B., and Aparicio, S. 1993. Characterization of the pufferfish (*Fugu*) genome as a compact vertebrate genome. Nature (London), **366**: 265–268.

Brooker, A.L., Cook, D., Bentzen, P., Wright, J.M., and Doyle, R.W. 1994. Organization of microsatellites differs between mammals and cold-water teleost fishes. Can. J. Fish. Aquat. Sci. **51**: 1959–1966.

Cargnelli, L., and Gross, M.R. 1996. The temporal dimension in fish recruitment: birth date, body size, and size-dependent survival in a sunfish (bluegill: *Lepomis macrochirus*). Can. J. Fish. Aquat. Sci. **53**: 360–367.

Coen, E.S., Thoday, J.M., and Dover, G. 1982. Rate of turnover of structural variants in the rDNA gene family of *Drosophila melanogaster*. Nature (London), **295**: 564–568.

Dallas, J.F. 1992. Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. Mamm. Genome, **3**: 452–456.

Estoup, A., Presa, P., Krieg, F., Vaiman, D., and Guyomard, R. 1993*a*. $(CT)_n$ and $(GT)_n$: a new class of genetic markers for *Salmo trutta* L. (brown trout). Heredity, **71**: 488–496.

Estoup, A., Solignac, M., Harry, M., and Cornuet, J.-M. 1993*b*. Characterization of $(GT)_n$ and $(CT)_n$ microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. Nucleic Acids Res. **21**: 1427–1431.

Fleming, I.A., and Gross, M.R. 1994. Breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*): measures of natural and sexual selection. Evolution, **48**: 637–657.

Goff, D.J., Galvin, K., Katz, H., Westerfield, M., Lander, E., and Tabin, C.J. 1992. Identification of polymorphic simple sequence repeats in the genome of the zebrafish. Genomics, **14**: 200–202.

Goode, B.L., and Feinstein, S.C. 1992. "Speedprep" purification of template for double-stranded DNA sequencing. BioTechniques, **12**: 374–375.

Gross, M.L., Kapuscinski, A.R., and Faras, A.J. 1994. Nest-specific DNA fingerprints of smallmouth bass in Lake Opeongo, Ontario. Trans. Am. Fish. Soc. **123**: 449–459.

Gross, M.R. 1982. Sneakers, satellites and parentals: polymorphic mating strategies in North American sunfishes. Z. Tierpsychol. **60**: 1–26.

Gross, M.R. 1984. Sunfish, salmon and the evolution of alternative reproductive strategies and tactics in fishes. *In* Fish reproduction: strategies and tactics. *Edited by* R.J. Wootton and G.W. Potts. Academic Press, London. pp. 55–75.

Gross, M.R. 1985. Disruptive selection for alternative life histories in salmon. Nature (London), **313**: 47–48.

Gross, M.R. 1991. Evolution of alternative reproductive strategies: frequency-dependent sexual selection in male bluegill sunfish. Philos. Trans. R. Soc. Lond. B Biol. Sci. **332**: 59–66.

Hedrick, P. 1994. Endangered Pacific salmonids. Conserv. Biol. **8**: 863–894.

Hutchings, J.A., and Myers, R.A. 1988. Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar*. Oecologia, **75**: 169–174.

Jarne, P., Viard, F., Delay, B., and Cuny, G. 1994. Variable microsatellites in the highly selfing snail *Bulinus truncatus* (Planorbidae: Basommatophoran). Mol. Ecol. **3**: 527–528.

Lewin, R. 1989. Limits to DNA fingerprinting. Science (Washington, D.C.), **243**: 1549–1551.

O'Reilly, P., and Wright, J.M. 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. J. Fish Biol. **47**(Suppl. A): 29–55.

Philipp, D.P., and Gross, M.R. 1994. Genetic evidence for cuckoldry in bluegill *Lepomis macrochirus*. Mol. Ecol. **3**: 563–569.

Queller, D.C., Strassmann, J.E., and Hughes, C.R. 1993. Microsatellites and kinship. Trends Ecol. Evol. **8**: 285–288.

Rico, C., Kuhnlein, U., and Fitzgerald, G.J. 1992. Male reproductive tactics in the threespine stickleback: an evaluation by DNA fingerprinting. Mol. Ecol. **1**: 79–87.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, New York.

Sanger, F., Nicklen, S., and Coulson, A.R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. **74**: 5463–5467.

Schroder, S.L. 1981. The role of sexual selection in determining overall mating patterns and mate choice in chum salmon. Ph.D. dissertation, University of Washington, Seattle.

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Fish. Res. Board Can. Bull. **184**: 1–966.

Slettan, A., Olsaker, I., and Lie, O. 1993. Isolation and characterization of variable $(GT)_n$ repetitive sequences from Atlantic salmon, *Salmo salar* L. Anim. Genet. **24**: 195–197.

Stallings, R.L., Ford, A.F., Nelson, D., Torney, D.C., Hildebrand, C.E., and Moyzis, R.K. 1991. Evolution and distribution of $(GT)_n$ repetitive sequences in mammalian genomes. Genomics, **10**: 807–815.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res. **17**: 6463–6471.

Tautz, D., and Renz, M. 1984. Simple sequences are ubiquitous components of eukaryotic genomes. Nucleic Acids Res. **12**: 4127–4138.

Weber, J.L. 1990. Informativeness of human $(dC\text{-}dA)_n\cdot(dG\text{-}dT)_n$ polymorphisms. Genomics, **7**: 524–530.

Weatherhead, P.J., and Montgomerie, R.D. 1991. Good news and bad news about DNA fingerprinting. Trends Ecol. Evol. **6**: 173–174.

Wintero, A.K., Fredholm, M., and Thomsen, P.D. 1992. Variable $(dG\text{-}dT)_n\cdot(dC\text{-}dA)_n$ sequences in the porcine genome. Genomics, **12**: 281–288.

Wirgin I.I., and Waldman, J.R. 1994. What DNA can do for you. Fisheries, **19**: 16–27.

Withler, R.E., and Beacham, T.D. 1994. Genetic consequences of the simultaneous or sequential addition of semen from multiple males during hatchery spawning of chinook salmon (*Oncorhyrchus tshawytscha*). Aquaculture, **126**: 11–23.

Wright, J.M. 1993. DNA fingerprinting of fishes. *In* Biochemistry and molecular biology of fishes. Vol. 2. *Edited by* P.W. Hochachka and T. Mommsen. Elsevier Press, New York. pp. 57–91.

Wright, J.M. 1994. Mutation at VNTRs: are minisatellites the evolutionary progeny of microsatellites? Genome, **37**: 1–3.

Wright, J.M., and Bentzen, P. 1994. Microsatellites: genetic markers for the future. *In* Reviews in fish biology and fisheries. Vol. 4. *Edited by* G.R. Carvalho and T.J. Pitcher. Chapman and Hall, London. pp. 384–388.

Spatial variation in recruitment, growth, and feeding of postsettlement King George whiting, *Sillaginodes punctata*, associated with seagrass beds of Port Phillip Bay, Australia

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Abstract: In the spring (October and November) of 1991, postsettlement individuals of King George whiting, *Sillaginodes punctata*, were collected from seagrass beds around the western coast of Port Phillip Bay using a fine-mesh beach seine. In October, immediately after most settlement had occurred, recruitment was negatively correlated with distance into the bay. By November, however, there was no correlation with distance from Port Phillip Heads. Recruitment was not correlated with seagrass biomass. Settlement hindcast from daily increments on otoliths indicated three settlement events, but only individuals from the first two settlement events reached the inner bay by late November. Growth of postsettlement individuals in the inner bay was significantly (20%) higher than it was nearer to Port Phillip Heads. In October, volume of prey in the diet was negatively correlated with recruitment, whilst in November prey volume was positively correlated with recruitment. We hypothesize that initial recruitment patterns were mainly the result of decreasing larval supply to the inner bay. Initial patterns were modified over time, possibly in response to variation in food supply.

Résumé : Au printemps de 1991 (octobre et novembre), on a recueilli, à l'aide d'une senne de rivage à petit maillage, des spécimens de merlan du Roi-George (*Sillaginodes punctata*) dans des herbiers près de la côte ouest de la baie Port Phillip. En octobre, immédiatement après la plus grande partie de la colonisation, le recrutement présentait une corrélation négative avec la distance dans la baie. Toutefois, vers le mois de novembre, il n'y avait plus de corrélation avec la distance de Port Phillip Heads; le recrutement n'était plus corrélé avec la biomasse des herbiers. Des prévisions à posteriori de la colonisation à partir d'incréments quotidiens sur les otolithes indiquaient qu'il y avait eu trois épisodes de colonisation, mais que seulement les individus des deux premiers épisodes avaient atteint la partie intérieure de la baie vers la fin de novembre. Le taux de croissance des individus de la période post-colonisation dans la partie intérieure de la baie était significativement plus élevé (20%) que plus près de Port Phillip Heads. En octobre, le volume des proies dans le régime alimentaire présentait une corrélation négative avec le recrutement, alors qu'en novembre, cette corrélation était positive. Pour expliquer ce phénomène, nous supposons que les profils initiaux du recrutement étaient causés surtout par l'approvisionnement décroissant en larves dans la portion intérieure de la baie. Les profils initiaux ont été modifiés avec le temps, peut-être en réponse à des variations de l'approvisionnement en nourriture.
[Traduit par la Rédaction]

Introduction

Seagrass is thought to be an important habitat for fish, including many of commercial value (Bell and Pollard 1989), and is extremely vulnerable to environmental degradation (Larkum et al. 1989). The management and preservation of fishes associated with seagrass depends to some extent on the processes that structure their communities (Bell et al. 1988). For example, if fish populations are mainly limited by the amount of cover (Heck and Orth 1980) then beds with highest seagrass density and (or) biomass should be preserved. However, if fish communities in seagrass beds are primarily limited by the availability of planktonic larvae (Bell et al. 1988) or postsettlement mortality independent of cover, then management depends on variability in recruitment. Under this scenario, some beds may receive consistently higher recruitment than others, and these beds should receive priority in management decisions. Alternatively, planktonic supply or postsettlement mortality may be unpredictable, requiring that seagrass beds be maintained in a range of sites, to maximize the chance of recruitment.

Older theory held that the diversity and abundance of seagrass fishes were determined by the amount of cover offered by seagrass as protection from predators (Heck and Orth

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1980). It follows that beds with higher biomass, density, complexity, or other measure of cover will harbour a greater diversity and abundance of fishes (Orth et al. 1984). Food was considered not to be a limiting factor, although this assumption has recently been called into question for macroinvertebrates in seagrass beds (Edgar 1990).

More recently, an alternative hypothesis (Bell and Westoby 1986) proposed that the abundance and diversity of fishes in a seagrass bed is a reflection of patchy larval input: larvae settle on the first seagrass bed they encounter, regardless of foliage characteristics. It follows from this hypothesis that, at the scale of individual seagrass beds, the habitat complexity hypothesis will hold; however, it will break down for disjunct beds at larger scales (i.e., tens of kilometres and greater) where community structure will be primarily determined by variability in larval settlement. Postsettlement individuals, however, may be considerably more mobile than first suggested by Bell and Westoby (1986). Sogard (1989) found that older postsettlement fishes may migrate over relatively large areas of unvegetated bottom to colonize artificial seagrass. Such movements would modify patterns initially produced by variation in larval supply. We define recruitment as the abundance of juveniles when sampled some arbitrary period after settlement, and therefore it is a function of both larval supply and postsettlement mortality and movement (Keough and Downes 1982). Orth (1992) has suggested that recruitment dynamics is a critical area of future research in seagrass ecology.

This paper describes the temporal and spatial pattern of recruitment of King George whiting, *Sillaginodes punctata*, to seagrass beds in Port Phillip Bay, Australia. The King George whiting is an important commercial species in southern Australian waters. Otoliths of postsettlement *S. punctata* contain daily increments that show a morphological transition related to environmental changes associated with entry to Port Phillip Bay (Jenkins and May 1994). Late-stage larvae, approximately 20 mm in length, enter Port Phillip Bay at an age of 100–170 d between September and November (Jenkins et al. 1993*b*; Jenkins and May 1994), apparently at the end of an extended and variable slow-growth phase where larvae would be competent to settle given a suitable environment (Jenkins and May 1994). The aims of the present paper are to (*i*) describe variation in recruitment over spatial scales greater than individual seagrass beds (tens of kilometres) and relate this to fine-scale temporal patterns of recruitment based on otolith daily increments, and seagrass biomass, at sites, and (*ii*) investigate spatial variation in feeding and growth of postsettlement *S. punctata*.

Methods

Study area

Port Phillip Bay is a large, semi-enclosed, predominantly tidal embayment linked to the ocean of Bass Strait by a narrow entrance (Fig. 1). The hydrodynamics are characterized by an entrance region where fast (3 $m \cdot s^{-1}$) ebb and flood jets dominate the circulation, a large flood-tidal delta, known as the Sands region, where strong currents occur in the major channels, and an inner zone where tidal currents are weak (Black et al. 1993). On the western side of Port Phillip Bay, tidal currents drop to below 10 $cm \cdot s^{-1}$ at about the entrance to the Geelong arm. Tides are semidiurnal and the range inside the bay is less than 1 m.

Seagrass around the Bellarine Peninsula area of Port Phillip Bay consists generally of narrow (20 m wide) bands of the subtidal seagrass *Heterozostera tasmanica* running parallel to the shoreline. Towards Corio Bay within the Geelong arm, however, seagrass beds become more widespread as a result of protection from the predominant southwesterly wind experienced in the region. This pattern reflects the size distribution of sediment grains, which become progressively finer from the Sands region around the Bellarine Peninsula and into the Geelong arm (Anonymous 1973). Salinity in the sampling area is essentially marine (Longmore et al. 1990).

Field sampling

Sampling encompassed 12 seagrass beds around a 50-km section of the Bellarine Peninsula (Fig. 1). *Heterozostera tasmanica* beds averaging approximately 0.5 m below mean low-water spring (MLWS) were sampled. Sampling was conducted on three occasions, September 30, October 27, and November 24, 1991. Five sites were sampled per day over 3 days, usually consecutively. Sampling was conducted over a 5-h period on either side of a near-midday low tide. The order of sampling sites was randomized as much as was practicable within logistical constraints.

Postsettlement *S. punctata* were sampled with a seine net of 10 m in length, a 3-m drop, and a mesh of approximately 1 mm^2. Two 10-m ropes were attached to each end of the seine. Four replicate hauls, haphazardly placed and nonoverlapping, were made at each site. Four replicates were considered sufficient to provide reasonable power on the basis of variation levels in previous sampling (Jenkins et al. 1993*b*). Hauls were made by two people walking out to the appropriate depth, with one person walking 10 m further, setting the net parallel to shore, and returning to the position of the second person where the net was then hauled into a bucket. The net was then carried back to shore where postlarvae and juveniles were sorted, anaesthetized in benzocaine, and preserved in 95% ethanol. A beach seine net is an efficient sampler of postsettlement *S. punctata* in seagrass habitat (Connolly 1994*a*).

Laboratory analysis

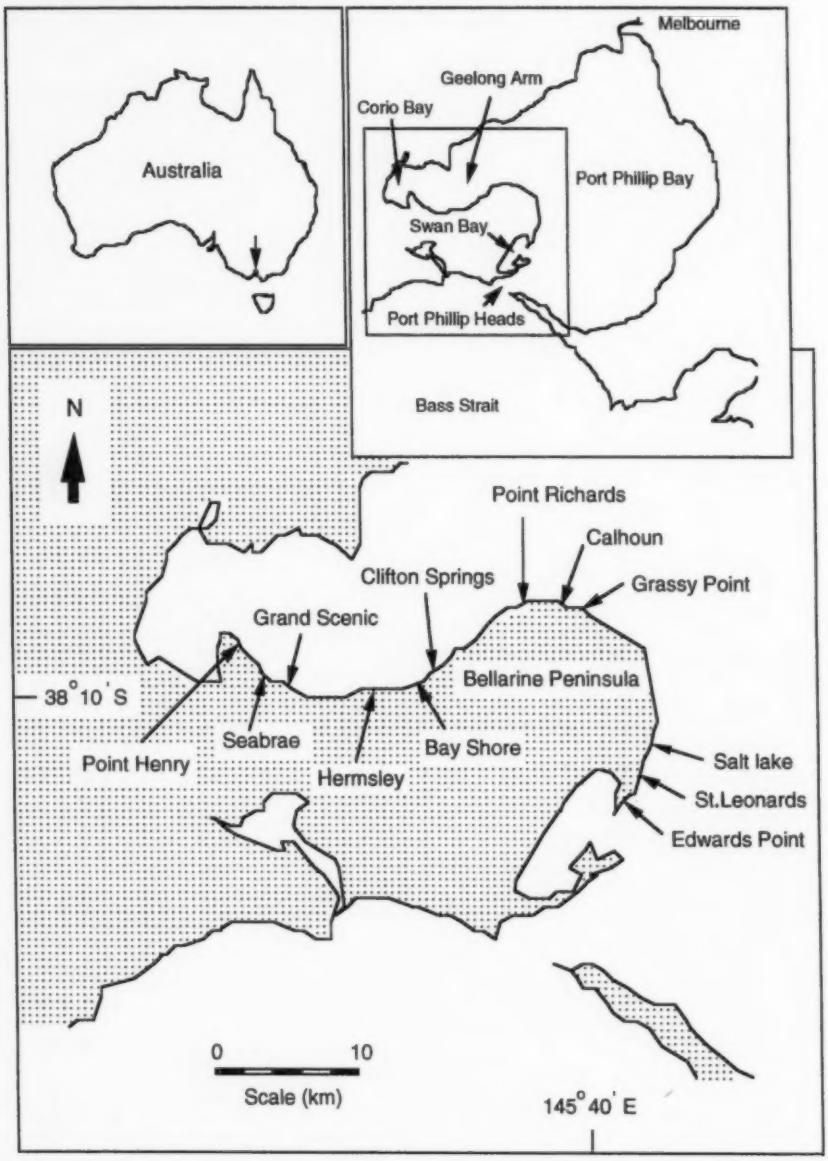
For samples with more than 20 specimens, a random subsample of 20 individuals was taken for analysis. The standard length (SL; tip of the snout to the tip of the caudal peduncle) of specimens was measured under a dissecting microscope fitted with an ocular micrometer. Specimens were placed in a drop of water on a microscope slide and the otoliths were dissected out using electrolytically sharpened tungsten needles. The daily periodicity of increment formation in *S. punctata* otoliths has been verified (Jenkins and May 1994). A transition in otolith microstructure, where increments show a marked increase in width at approximately the time of arrival of larvae in Port Phillip Bay, was used to backcalculate the approximate date of settlement of individuals (Jenkins and Black 1994; Jenkins and May 1994).

After otoliths were removed, specimens were analyzed for dietary composition and volume of stomach contents. Specimens were placed in a drop of glycerine on a microscope slide, and while they were being viewed under a stereomicroscope, tungsten needles were used to tease prey items from the alimentary canal. The entire alimentary canal, which in the early postsettlement stage forms a relatively straight tube, was examined. All prey items were identified to the lowest taxonomic level possible and counted. In most cases prey items were intact; however, where fragmentation had occurred only heads, where identifiable, were counted. A piece of 1-mm^2 grid graph paper was placed under the slide and an index of gut volume was determined from the area covered by gut contents.

Seagrass biomass

Above-ground biomass of seagrass was sampled on December 12, 1991. A 25×25 cm quadrat was used to take five haphazardly placed, replicate samples at each site. The stems at the sediment interface within the quadrat were cut with scissors. Samples were placed on

Fig. 1. Location of sampling sites around the Bellarine Peninsula region of Port Phillip Bay, Victoria, from which juvenile fish were collected. Insets: Location of the Bellarine Peninsula in Port Phillip Bay and location of Port Phillip Bay on the Australian coast.



ice for transport to the laboratory where they were dried for 2 days at 60°C and weighed.

Data analysis

Assumptions of analysis of variance were examined using box and residual plots. For the analysis of recruitment, site was considered a random factor. Correlation of fish abundance with distance around the coast from Port Phillip Heads, seagrass biomass, and feeding indices was determined using the Pearson correlation coefficient. In all cases, transformation to $\log(x + 1)$ greatly improved the homogeneity of the variances and the distribution of residuals. Ryan's Q test was used for post-hoc comparisons after ANOVA (Day and Quinn 1989). Growth rates, determined from postsettlement size at age, were compared using analysis of covariance.

Results

Recruitment

In September, King George whiting recruits were collected only from the Calhoun site (Fig. 2). Analysis of variance produced a significant interaction between sampling date and site

Fig. 2. Mean abundance of postsettlement *S. punctata* in seine-net samples from sites around the Bellarine Peninsula over three sampling dates. Error bars are standard error.

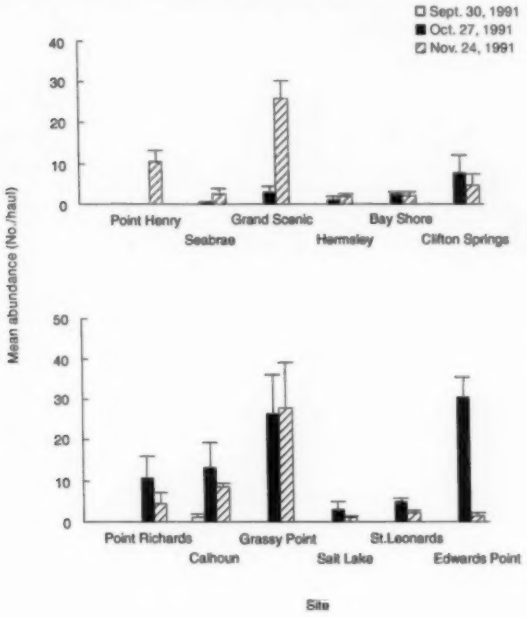


Fig. 3. Mean seagrass biomass from sites around the Bellarine Peninsula sampled in December 1991. PH, Point Henry; S, Seabrae; GS, Grand Scenic; H, Hermsley; BS, Bay Shore; CS, Clifton Springs; PR, Point Richards; C, Calhoun; GP, Grassy Point; SL, Salt Lake; StL, St. Leonards; EP, Edwards Point. Error bars are standard error.

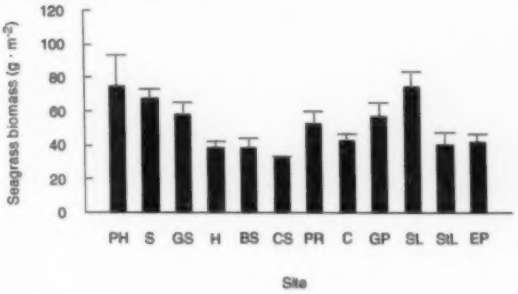


Table 1. Analysis of variance of postsettlement King George whiting abundances at 12 sites around the Bellarine Peninsula on two sampling dates.

Source	df	MS	*F*	*P*
Date	1	0.002	0.001	ns
Site	11	4.533	9.785	<0.001
Date × site	11	3.761	8.118	<0.001
Error	72	0.463		

Note: df, degrees of freedom; MS, mean square; ns, not significant.

for October and November sampling (Table 1). Recruitment in October was highest at the sites near the entrance to the Geelong arm and at Edwards Point, and lowest at sites furthest into the bay (Fig. 2). This was supported by post-hoc testing which showed that the Edwards Point, Grassy Point, Calhoun, and Point Richards sites had significantly higher abundances than the Hermsley, Seabrae, and Point Henry sites. In late November the level of recruitment had remained relatively high at sites near the entrance to the Geelong arm (Fig. 2). Recruitment increased significantly at the Point Henry and Grand Scenic sites deep inside the bay and decreased significantly at the Edwards Point site, resulting in uniformly low abundances at sites near Port Phillip Heads (Fig. 2). Overall, abundances at the Grassy Point, Calhoun, Grand Scenic, and Point Henry sites were significantly higher than at the Salt Lake and Edwards Point sites.

In October, there was a negative correlation between recruitment and distance around the coast from Port Phillip Heads ($n = 12$, $r = -0.75$, $P = 0.005$), but this was not so by late November ($n = 12$, $r = 0.31$, $P = 0.32$).

Seagrass biomass

Seagrass biomass per unit area (Fig. 3) was different amongst sites (ANOVA df = 11/48, $F = 4.084$, $P = 0.0003$). There was no correlation between seagrass biomass and recruitment in October ($n = 12$, $r = -0.37$, $P = 0.23$) or November ($n = 12$, $r = 0.29$, $P = 0.36$).

Length frequencies

In October, the smallest *S. punctata* recruits were collected at Edwards Point and St. Leonards, ranging from 18 to 22 mm with a mode of approximately 20 mm (Fig. 4). From Grassy Point to Point Richards, sizes ranged from 20 to 25 mm with a mode between 21 and 22 mm (Fig. 4). A similar size range occurred between Clifton Springs and Grand Scenic; however, the mode was closer to 23 mm (Fig. 4). In November, recruits from Edwards Point to Salt Lake were all less than 30 mm SL, and included small individuals of 20 mm or less (Fig. 4). A few small (approximately 20 mm) individuals were present between Grassy Point and Point Richards in November; however, most ranged from 24 to 33 mm in length (Fig. 4). Recruits collected in November between Clifton Springs and Point Henry were relatively large, ranging from approximately 25 to 37 mm SL (Fig. 4).

Otolith analysis

Otoliths from *S. punctata* recruits collected in late September at the Calhoun site had between one and three post-transition increments. Overall, *S. punctata* recruits sampled in October and November were derived from three major cohorts with otolith transitions between days of the year 272–282, 285–292, and 294–305, respectively (Fig. 5). The cohorts were not represented evenly amongst the sites. In October, recruits at sites between Grand Scenic and Clifton Springs came predominantly from the earliest cohort, with no recruits from the third cohort. All three cohorts were represented at sites between Point Richards and Edwards Point; however, the first cohort still dominated. The St. Leonards site had equal representation from the first and third cohorts while at Edwards Point the third cohort dominated. The otolith transition for recruits in the third cohort was formed only a few days

Fig. 4. Length frequency distributions for postsettlement *S. punctata* collected in October (solid bars) and November (open bars).

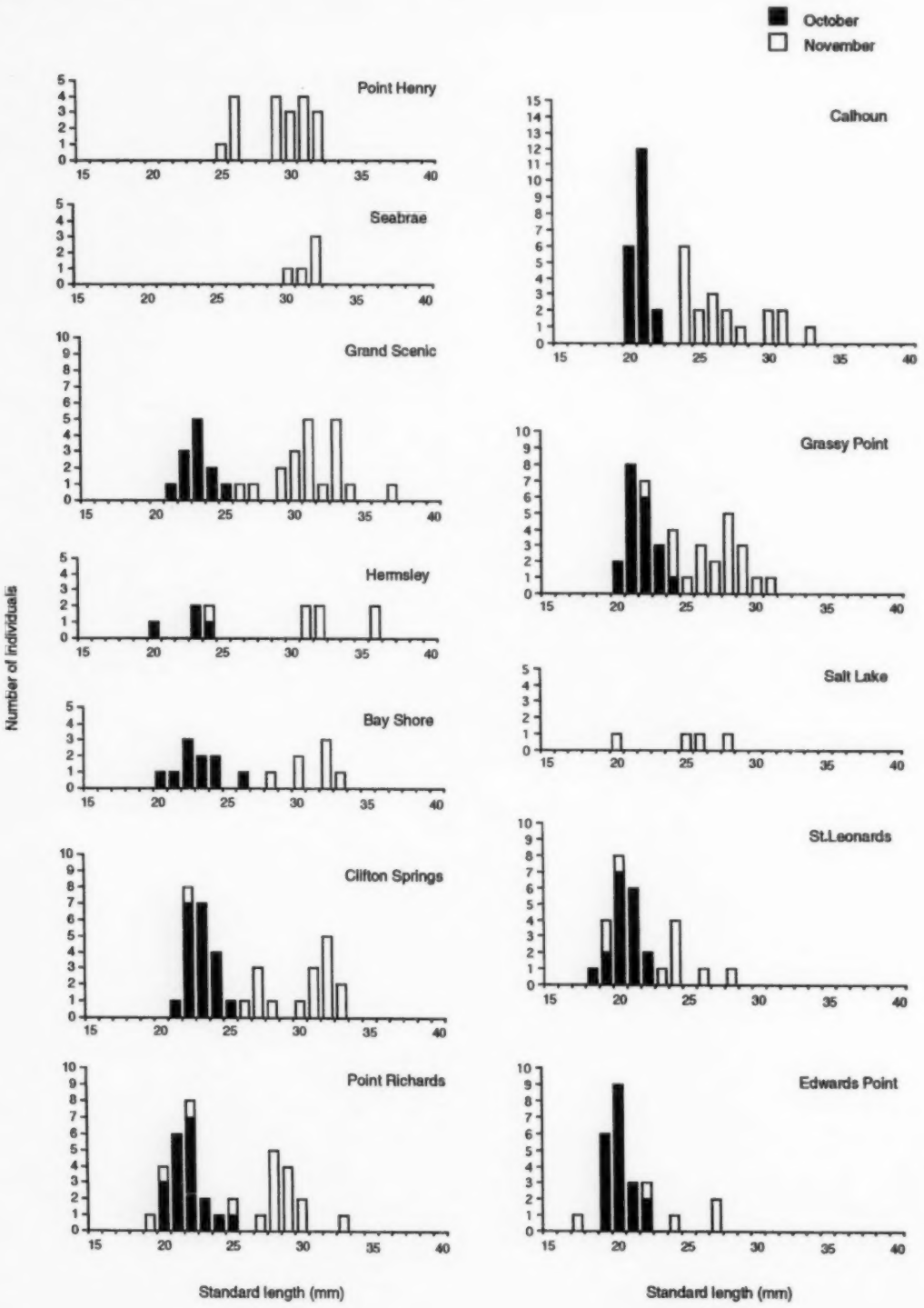


Fig. 5. Back-calculated date of otolith transition for postsettlement *S. punctata* collected in October (solid bars) and November (open bars).

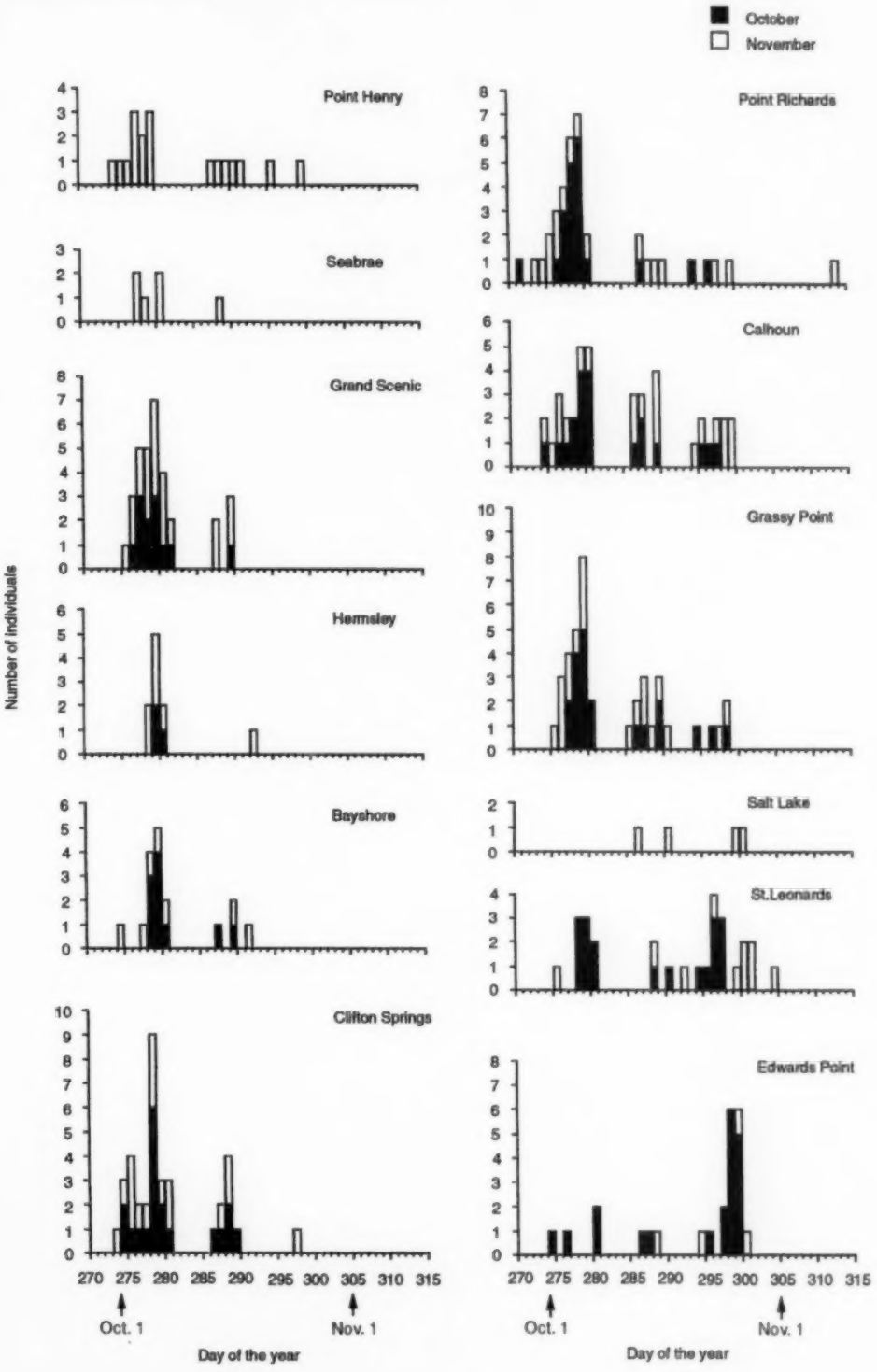


Fig. 6. Diet of postsettlement *S. punctata* collected in October. (A) Mean number and composition of prey items. (B) Mean volume of prey items. Error bars are standard error.

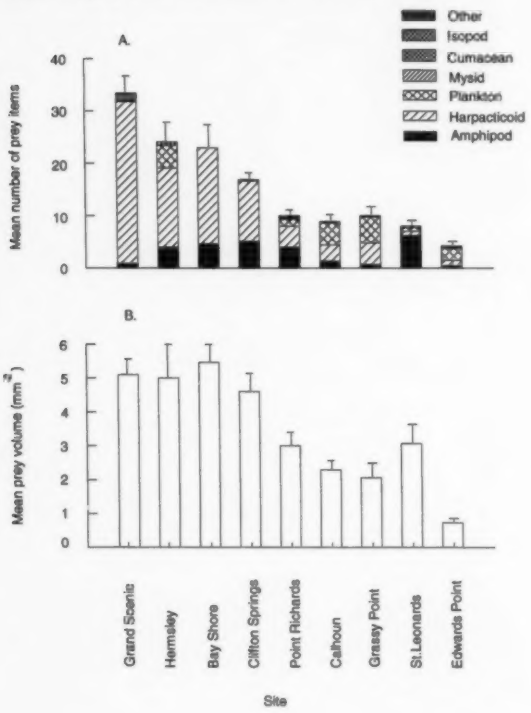
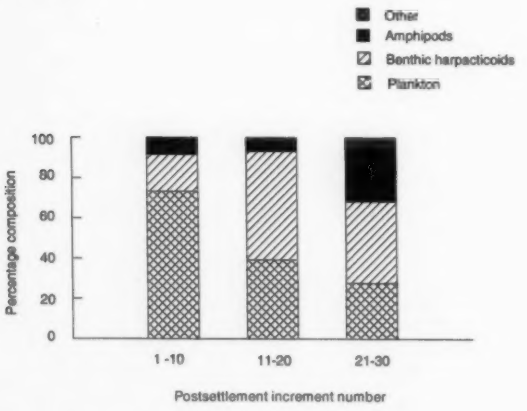
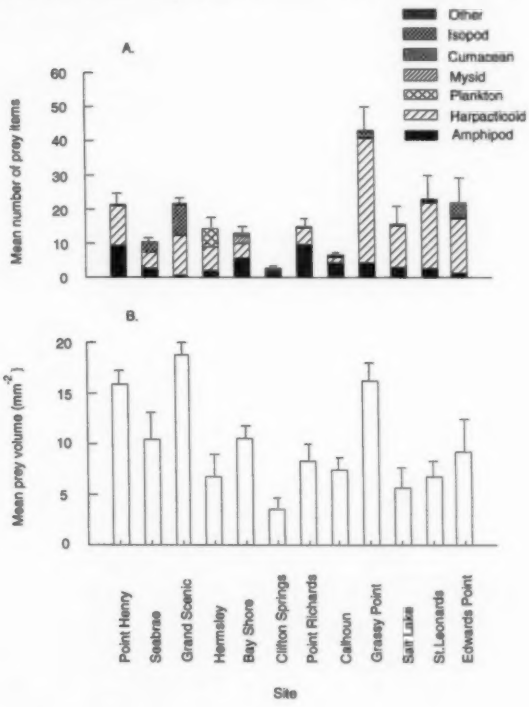


Fig. 7. Percentage composition of prey items in the diet of postsettlement *S. punctata* from the Portarlington region in October.



before sampling in October. Overall, the increasing dominance of the earliest cohort with distance into the bay explained the corresponding increase in recruit size along the same gradient (Fig. 4). In November the pattern was similar; sites in the Geelong arm between Point Henry and Clifton Springs were still dominated by the earliest cohort with lower numbers of the second cohort, although a few individuals from

Fig. 8. Diet of postsettlement *S. punctata* collected in November. (A) Mean number and composition of prey items. (B) Mean volume of prey items. Error bars are standard error.



the third cohort occurred at Point Henry and Clifton Springs. There was a more even representation of the cohorts at the entrance to the Geelong arm between Point Richards and Grassy Point compared with the dominance of the first cohort in October. Only one individual from the first cohort remained in the samples from Salt Lake to Edwards Point in November. Once again, the increasing dominance of the earlier cohorts with distance into the bay was reflected in the increasing size along this gradient (Fig. 4).

Feeding

In October there was a trend toward increasing mean number of prey items (Fig. 6A) and prey volume in the gut (Fig. 6B) with distance into the bay. Prey items were dominated by benthic harpacticoid copepods, gammaridian amphipods, and at the Calhoun, Grassy Point, and Edwards Point sites, planktonic Crustacea (Fig. 6A). For individuals from the Point Richards, Calhoun, and Grassy Point sites, collected in October, where all three cohorts were represented (Fig. 5), there was a decreasing proportion of plankton in the diet with increasing post-transition age (Fig. 7). Moreover, in this region there was a positive correlation between prey volume and post-transition increment number ($n = 63$, $r = 0.26$, $P = 0.038$). Thus, the increased proportion of planktonic Crustacea in the diet and lower prey volume at sites nearer to Port Phillip Heads related in part to the presence of the third cohort, which consisted of more recently recruited individuals. In October there

Table 2. Regression equations and analysis of variance for the relationship between standard length and post-transition increment number on otoliths of juvenile *S. punctata* from four regions.

Region	Slope (mm·d^{-1})	Intercept (mm)	r^2	n	MS	F	P
Corio	0.304±0.039	15.242	0.83	52	587.52	246.90	<0.001
Clifton	0.311±0.037	14.901	0.84	55	910.42	277.95	<0.001
Portarlington	0.242±0.020	16.420	0.88	86	959.37	618.52	<0.001
St. Leonards	0.244±0.059	15.639	0.83	18	105.05	77.85	<0.001

Note: Slopes are given as the mean ± 95% confidence intervals.

Table 3. Analysis of covariance of standard length amongst regions with post-transition increment number on otoliths as the covariate.

Source	df	MS	F	P
Region	3	3.25	1.49	ns
Increments	1	1359.07	620.74	<0.001
Region × increments	3	12.09	5.52	<0.001
Error	203	2.19		

Note: df, degrees of freedom; MS, mean square; ns, not significant.

was a negative correlation between prey volume and recruitment among sites ($N = 9$, $r = -0.89$, $P = 0.001$).

In November, feeding patterns were quite different. The mean number of prey items per individual was quite variable with distance from Port Phillip Heads, with highest numbers at Grassy Point (Fig. 8A). The pattern for prey volume was similar, although the volumes at the Point Henry, Seabrae, and Grand Scenic sites were relatively higher compared with other sites than were prey numbers (Fig. 8B). The inconsistency between prey number and prey volume in the case of the Seabrae and Grand Scenic sites was related to the consumption of relatively large isopods (Fig. 8A). In November there was a positive correlation between prey volume and recruitment at sites ($N = 12$, $r = 0.78$, $P = 0.003$).

Growth

It was necessary to pool data from sites into four regions to obtain sufficient sample sizes for analysis. These regions were Corio (Point Henry, Seabrae, and Grand Scenic), Clifton (Hermsley, Bay Shore, and Clifton Springs), Portarlington (Point Richards, Calhoun, and Grassy Point), and St. Leonards (Salt Lake, St. Leonards, and Edwards Point). Growth was compared amongst regions using size at postsettlement age (Jenkins and May 1994). Data from the October and November sampling were pooled. For a direct comparison to be made, data were restricted to individuals from the first two cohorts (settlement days 270–292) and with between 20 and 60 post-transition otolith increments. Growth rate was approximately linear over this range of increments.

The growth rate in the Corio and Clifton regions of approximately 0.3 mm·d^{-1} was 20% higher than in the Portarlington and St. Leonards regions where the growth rate was approximately 0.25 mm·d^{-1} (Table 2). The relatively wide confidence limits on the growth rate estimate for the St. Leonards region may have partially resulted from the small sample size (Table 2). The interaction term in an analysis of variance of SL amongst regions with post-transition increment number as a covariate indicates that the regional differences in growth rate were significant (Table 3). Thus, higher growth rates in the inner bay would have contributed to the relatively larger size of recruits than at sites nearer the entrance (Fig. 4).

Discussion

Seagrass biomass appeared to have little influence on recruitment levels of *S. punctata*. This result is consistent with the hypothesis of Bell and Westoby (1986), which suggests that the correlations with habitat structure often observed within seagrass beds will tend to break down at larger scales because of the patchy distribution of recruiting larvae. Biomass is only one characteristic of seagrass habitat; others such as shoot density and shoot length were not considered. Recent deployment of artificial seagrass (G.P. Jenkins, unpublished data) around the Bellarine Peninsula supports the contention that spatial patterns in recruitment in the present study are not related to habitat structure. Postsettlement *S. punctata* have a complex relationship with seagrass habitat. Individuals show a preference for seagrass in Port Phillip Bay but in Swan Bay (Fig. 1) occur mainly on unvegetated patches (approximately 5–20 m in diameter) within expansive beds of subtidal *H. tasmanica* (Jenkins et al. 1993*b*).

Larval supply might be expected to have a major influence on spatial patterns of recruitment in *S. punctata* given the strong hydrodynamic influence on the temporal pattern of recruitment already detected in recruitment data from 1989 (Jenkins and Black 1994). Like the present study, Jenkins and May (1994) found that otolith analysis suggested an approximate 7- to 10-d cycle of larvae arriving at juvenile habitats in Port Phillip Bay. A model of advection of passive particles from Bass Strait to Port Phillip Bay over the same period showed a significant correlation between particle entry and the timing of otolith transition (Jenkins and Black 1994). This cycle was related to a combination of climatic and hydrodynamic factors that influenced the net flow of water to Port Phillip Bay (Jenkins and Black 1994). As in this study, the smallest recruits collected had only one or two post-transition increments, suggesting that this transition was closely tied to the arrival of larvae in Port Phillip Bay. Because model predictions and otolith data were correlated at zero lag, Jenkins and Black (1994) suggested that the otolith transition was more related to entry to Port Phillip Bay than to arrival at a specific site. Our data indicate that presettlement larvae can have post-transition increments (G.P. Jenkins, unpublished data), suggesting that the otolith transition may be related to changed conditions of temperature and food supply upon entry to Port Phillip Bay, rather than the conditions at a particular site (Jenkins and May 1994). One possibility is that most larvae entering Port Phillip Bay when hydrodynamic conditions are suitable arrive at nearshore seagrass beds fairly quickly,

but some may stay in the plankton and arrive at the juvenile habitat at a later time. It is also possible that larvae may 'bounce', settling temporarily but reentering the plankton (Rijnsdorp et al. 1985), until a suitable habitat is found.

Larval supply may partly explain the distribution of recruits around the coast of Port Phillip Bay. There was significantly lower recruitment to sites furthest into Port Phillip Bay in October. Only two of the three main cohorts were represented at sites within the Geelong arm in October, whilst the third cohort, which had apparently recruited a few days before sampling, was also represented at sites nearer Port Phillip Heads. The major influence of strong tidal currents associated with Port Phillip Heads declines with distance into the bay to low levels (less than 10 $cm \cdot s^{-1}$) at approximately the entrance to the Geelong arm (Black et al. 1993). Modelling of passive-particle entry to Port Phillip Bay over the *S. punctata* recruitment period in spring 1989 showed that, at least in the vertically averaged case, particles did not penetrate further into the bay than the entrance to the Geelong arm (Jenkins and Black 1994). The hypothesis that larval supply is limiting recruitment to the inner regions of the bay could be tested by plankton sampling at multiple sites around the coast, including the inner bay, to determine the extent of larval transport. High frequency sampling of larvae and postsettlement individuals will be required to examine the relationship between larval supply and settlement in detail.

The pattern of decreasing recruitment with distance from the bay entrance in October was disrupted by low recruitment at the St. Leonards and Salt Lake sites in the region closest to Port Phillip Heads. All sites in this region showed low recruitment in November. This pattern is not necessarily inconsistent with higher larval supply towards Port Phillip Heads. The presence of only recently recruited individuals in this region may suggest a high rate of postsettlement mortality (Sale and Ferrell 1988; Booth 1991; Connell and Jones 1991; Levin 1994) or migration (Sogard 1989). Alternatively, a passive mechanism may be involved where nearshore current strengths near Port Phillip Heads are too strong for larvae to maintain themselves in seagrass beds. A possible explanation for the high abundance of recently settled individuals at Edwards Point in October, compared with the low abundances at nearby sites, is that strong currents and wave action tend to resuspend and transport settlers through the area, causing a patchy distribution in space and time. By November, there was no consistent trend in recruitment with distance from Port Phillip Heads. The finding that there were high numbers of individuals from the first two recruitment cohorts in the innermost region in November, whereas there were very low numbers of recruits in the region in October, suggests that significant migration of juveniles may have occurred (Sogard 1989).

Edgar (1990) has postulated that food limitation may be an important factor structuring epifaunal communities in seagrass. In the case of fishes associated with seagrass, however, food availability has received little consideration as a possible factor influencing postsettlement mortality or migration. In our study, the negative correlation between food volume and recruitment in October could be attributable to high recruitment in areas of low food production near Port Phillip Heads (Shaw and Jenkins 1992) or perhaps to density-dependent competition for food (Jenkins et al. 1991). Another possible factor may be that recruits from sites between Port Phillip Heads and the entrance to the Geelong arm included newly settled individuals with lower volumes of food in the gut than older individuals. By November, however, there was a positive correlation between prey volume and recruitment, suggesting that individuals were aggregating, or surviving better, in areas with greater food resources. A caveat that must be applied to these results is that gut content data represent only recent feeding conditions and may be affected strongly by factors such as time of sampling. The importance of food in determining the distribution patterns and habitat utilization of *S. punctata* has also been emphasised by Connolly (1994*b*, 1994*c*) in laboratory and field experiments.

The initial growth rate of postsettlement *S. punctata* is very slow, at approximately 0.05 $mm \cdot d^{-1}$, after which it gradually increases (Jenkins and May 1994). Growth rate was significantly higher in the Geelong arm than it was closer to Port Phillip Heads. This may have partly resulted from higher water temperatures in the inner bay, which warms more rapidly over the spring period than areas closer to Port Phillip Heads that are influenced by Bass Strait circulation (Longmore et al. 1990). Additionally, the more protected sites within the Geelong arm may have a higher production of meiofaunal food than the more exposed sites nearer to Port Phillip Heads because a greater proportion of seagrass debris would be retained in situ and feed into the detrital food chain (Shaw and Jenkins 1992; Jenkins et al. 1993*a*). The higher prey volumes for postsettlement *S. punctata* from the inner bay in October may partly reflect such a process.

In conclusion, levels of recruitment to seagrass beds in Port Phillip Bay were not predictable from knowledge of the seagrass biomass. The most parsimonious hypothesis to explain the distribution would be the combined effects of a limit on larval supply to the inner bay owing to the characteristics of circulation, together with postsettlement mortality, migration, or possibly an inability of recruits to maintain position in the face of strong tidal currents in the area close to Port Phillip Heads. This would lead to maximal initial recruitment to the area at the entrance to the Geelong arm. Data on feeding and growth suggest that food availability may be an important factor in postsettlement mortality and movement in this species. Expanded sampling of plankton and artificial seagrass, together with localized high frequency sampling, is presently underway to investigate these ideas. Finally, the recruitment pattern described in this study has shown some consistency over 3 different years of sampling (G.P. Jenkins, unpublished data), suggesting that factors influencing recruitment are reasonably stable over time and may allow for the protection of critical areas such as at the entrance to the Geelong arm, which would not have been identified simply on the basis of habitat structure.

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References

Anonymous. 1973. Environmental study of Port Phillip Bay. Report on phase one, 1968–1971. Melbourne and Metropolitan Board of Works, and Fisheries and Wildlife Department of Victoria, Melbourne, Australia.

Bell, J.D., and Pollard, D.A. 1989. Ecology of fish assemblages and fisheries associated with seagrasses. *In* Biology of seagrasses: a treatise on the biology of seagrasses with special reference to the Australian region. *Edited by* A.W.D. Larkum, A.J. McComb, and S. Shepherd. Elsevier, Amsterdam. pp. 565–609.

Bell, J.D., and Westoby, M. 1986. Variation in seagrass height and density over a wide spatial scale: effects on common fish and decapods. J. Exp. Mar. Biol. Ecol. **104**: 275–295.

Bell, J.D., Steffe, A.S., and Westoby, M. 1988. Location of seagrass beds in estuaries: effects on associated fish and decapods. J. Exp. Mar. Biol. Ecol. **122**: 127–146.

Black, K.P., Hatton, D., and Rosenberg, M. 1993. Locally and externally-driven dynamics of a large semienclosed bay in southern Australia. J. Coastal Res. **9**: 509–538.

Booth, D.J. 1991. The effects of sampling frequency on estimates of recruitment of the domino damselfish *Dascyllus albisella* Gill. J. Exp. Mar. Biol. Ecol. **145**: 149–159.

Connell, S.D., and Jones, G.P. 1991. The influence of habitat complexity on postrecruitment processes in a temperate reef fish population. J. Exp. Mar. Biol. Ecol. **151**: 271–294.

Connolly, R.M. 1994*a*. Comparison of fish catches from a buoyant pop net and a beach seine net in a shallow seagrass habitat. Mar. Ecol. Prog. Ser. **109**: 305–309.

Connolly, R.M. 1994*b*. Removal of seagrass canopy: effects on small fish and their prey. J. Exp. Mar. Biol. Ecol. **184**: 99–110.

Connolly, R.M. 1994*c*. The role of seagrass as preferred habitat for juvenile *Sillaginodes punctata* (Cuv. & Val.) (Sillaginidae, Pisces): habitat selection or feeding? J. Exp. Mar. Biol. Ecol. **180**: 39–47.

Day, R.W., and Quinn, G.P. 1989. Comparison of treatments after an analysis of variance in ecology. Ecol. Monogr. **59**: 433–463.

Edgar, G.J. 1990. Population regulation, population dynamics and competition amongst mobile epifauna associated with seagrass. J. Exp. Mar. Biol. Ecol. **144**: 205–234.

Heck, K.L., Jr., and Orth, R.J. 1980. Seagrass habitats: the roles of habitat complexity, competition and predation in structuring associated fish and motile macroinvertebrate assemblages. *In* Estuarine perspectives. *Edited by* V.S. Kennedy. Academic Press Inc., New York. pp. 449–464.

Jenkins, G.P., and Black, K.P. 1994. Temporal variability in settlement of a coastal fish, the King George whiting, *Sillaginodes punctata*, is determined by low-frequency hydrodynamics. Limnol. Oceanogr. **39**: 1744–1754.

Jenkins, G.P., and May, H.M.A. 1994. Variation in settlement and larval duration of King George whiting, *Sillaginodes punctata* (Sillaginidae), in Swan Bay, Victoria, Australia. Bull. Mar. Sci. **54**: 281–296.

Jenkins, G.P., Young, J.W., and Davis, T.L.O. 1991. Density dependence of larval growth of a marine fish, the southern bluefin tuna, *Thunnus maccoyii*. Can. J. Fish. Aquat. Sci. **48**: 1358–1363.

Jenkins, G.P., Shaw, M., and Stewart, B.D. 1993*a*. Spatial variation in food-limited growth of juvenile greenback flounder, *Rhombosolea tapirina*: evidence from otolith daily increments and otolith scaling. Can. J. Fish. Aquat. Sci. **50**: 2558–2567.

Jenkins, G.P., Watson, G.F., and Hammond, L.S. 1993*b*. Patterns of utilisation of seagrass (*Heterozostera*) dominated habitats as nursery areas by commercially important fish. *In* Victorian Institute of Marine Sciences. Vol. 19.

Keough, M.J., and Downes, B.J. 1982. Recruitment of marine invertebrates: the role of active larval choices and early mortality. Oecologia, **54**: 348–352.

Larkum, A.W.D., McComb, A.J., and Shepherd, S.A. 1989. Biology of seagrasses. Elsevier, Amsterdam.

Levin, P.S. 1994. Fine-scale temporal variation in recruitment of a temperate demersal fish: the importance of settlement versus post-settlement loss. Oecologia, **97**: 124–133.

Longmore, A.R., Cowdell, R.A., and Gibbs, C.F. 1990. Monitoring Port Phillip Bay water quality 1985–86. Sci. Rep. Ser. No. 89/003. Environmental Protection Authority, Melbourne, Australia.

Orth, R.J. 1992. A perspective on plant–animal interactions in seagrasses: physical and biological determinants influencing plant and animal abundance. *In* Plant–animal interactions in the marine benthos. Systematics Association Spec. Vol. No. 46. *Edited by* D.M. John, S.J. Hawkins, and J.H. Price. Clarendon Press, Oxford. pp. 147–164.

Orth, R.J., Heck, K.L., and Van Montfran, J.S. 1984. Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator–prey relationships. Estuaries, **7**: 339–350.

Rijnsdorp, A.D., Van Stralen, M., and Van der Veer, H.W. 1985. Selective tidal transport of North Sea plaice larvae *Pleuronectes platessa* in coastal nursery areas. Trans. Am. Fish. Soc. **114**: 461–470.

Sale, P.F., and Ferrell, D.J. 1988. Early survivorship of juvenile coral reef fishes. Coral Reefs, **7**: 117–124.

Shaw, M., and Jenkins, G.P. 1992. Spatial variation in feeding, prey distribution and food limitation of juvenile flounder *Rhombosolea tapirina* Gunther. J. Exp. Mar. Biol. Ecol. **165**: 1–21.

Sogard, S.M. 1989. Colonization of artificial seagrass by fishes and decapod crustaceans: importance of proximity to natural eelgrass. J. Exp. Mar. Biol. Ecol. **133**: 15–37.

The temporal dimension in fish recruitment: birth date, body size, and size-dependent survival in a sunfish (bluegill: *Lepomis macrochirus*)

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Abstract: Many fish species are protracted spawners, producing fry over a several-week breeding season. We demonstrate that this time dimension has important implications for recruitment by relating the body size and survival of yearlings to the day that they were born. The production of bluegill sunfish fry was estimated throughout the 1993 spawning season in Lake Opinicon, Ontario. In 1994, yearlings were collected and otolith daily growth rings used to determine their 1993 date of birth. We found that yearling body size decreased with date of birth in 1993. There was a progressive decline in body size across the eight spawning bouts, resulting in a 50% difference in the size at age 1 of early- and late-born fry. This difference is apparently due to the longer growing season available to early-born fry. Fry born earlier in the season also had strikingly higher survivorship to age 1 than fry from the middle- or late-season bouts. Fry produced during the first trimester increased 231% in representation among yearlings. This increased survivorship is probably due to increased over-winter survivorship and possibly also size-selective predation. These results highlight the importance of the temporal dimension in understanding the nature of fish recruitment.

Résumé : De nombreuses espèces de poissons ont une saison de fraye relativement longue, de sorte que les alevins naissent sur une période de plusieurs semaines. Nous démontrons que cette dimension temporelle a des implications importantes pour le recrutement en établissant un rapport entre d'une part la taille corporelle et le taux de survie des poissons d'un an, et d'autre part le jour de leur naissance. On a estimé la production d'alevins de crapet arlequin pendant toute la saison de fraye de 1993 dans le lac Opinicon (Ontario). En 1994, on a prélevé des poissons d'un an et on a utilisé les anneaux de croissance quotidienne des otolithes pour déterminer la date de leur naissance (en 1993). On a observé un déclin progressif de la taille corporelle en fonction de la date de naissance en 1993, soit à la suite de huit épisodes de fraye, qui entraînait à 1 an une différence de taille de 50% entre les alevins nés au début et à la fin de la saison de fraye. Cette différence est apparemment due à la saison de croissance plus longue dont pouvaient profiter les alevins nés au début de la période. De plus, on observait chez ces derniers un taux de survie à 1 an notablement plus élevé par rapport à celui des alevins nés au milieu ou à la fin de la saison. La représentation des alevins nés au cours du premier trimestre a augmenté de 231% dans le groupe des poissons d'un an. Cette augmentation du taux de survie est probablement due à un taux de survie accru après hivernage et peut-être aussi à des facteurs de prédation dépendant de la taille. Ces résultats soulignent l'importance de la dimension temporelle des études visant à améliorer la compréhension du processus de recrutement des poissons.
[Traduit par la Rédaction]

Introduction

Recruitment, the addition of new members to a population through reproduction (Fogarty et al. 1991), is a central issue in fish population biology. Recruitment, and the variability associated with it, affects not only population size, but also population structure, stability, and genetic composition. Recent research has indicated that the early life history stages are critical to determining adult population sizes, and a number of important processes that influence recruitment and year-class strength have been identified, including size-selective predation (Post and Prankevicius 1987; Post and Evans 1989*a*; Litvak and Leggett 1992), size-dependent feeding and starvation (Miller et al. 1988), and size-dependent over-winter mortality (Oliver et al. 1979; Toneys and Coble 1979; Shuter et al. 1980; Post and Evans 1989*b*; Smith and Griffith 1994). These studies show that survivorship can be biased towards larger individuals in a population (for an exception see Litvak and Leggett 1992).

An important dimension to survival has not been previously addressed. No study has related body size and survival to actual spawning times. Many temperate fish species have breeding seasons that span a substantial portion of the summer growing season. For example, about 74% of Canadian freshwater fish species have breeding seasons that extend for more than 1 month (n = 86 species from 26 families; data in Scott and Crossman 1973). This temporal dimension within a season may result in a wide range of birth dates and potential growth opportunities, and may be important to understanding recruitment and year-class strength of temperate fish populations.

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In this study, we examine within-season temporal variation in recruitment, yearling body size, and survival of young-of-the-year bluegill sunfish, *Lepomis macrochirus*. We link survival to age 1 with individual birth dates from the previous year. We find that early-born individuals attain the largest body size and have the highest survivorship to age 1. We discuss the implications of these results for recruitment theory, year-class strength, and the size structure of temperate fish populations.

Methods

Bluegill reproductive biology

The bluegill sunfish is a synchronously breeding, colonial centrarchid found in eastern and central North America (Scott and Crossman 1973). In Lake Opinicon, the breeding season ranges from as early as 21 May to as late as 24 July (data from 1985–1993). Spawning is initiated by males entering the littoral zone and building nests in the substrate (Gross 1980, 1982). Colonies, varying in size from 1 to 300 nests, are formed by parental males in water 0.5–3.5 m deep. These males range in age from 7 to 11 years. Gravid females, 4–7 years of age, approach male colonies in schools and individually enter nests where they spawn with parental males. When spawning is complete, females leave the colony and retreat to deeper waters while parental males remain on their nests to care for the embryos in their nests. Depending on water temperatures, fry reach swim-up in 5–12 days and then the parental male abandons the nest (L.M. Cargnelli and M.R. Gross, University of Toronto, unpublished data).

Spawning occurs in distinct bouts, in which a number of colonies form and spawn synchronously. There are at least several days of colony inactivity before the next spawning bout. As a result, all colonies formed during a bout are at approximately the same stage of development. The number of spawning bouts during a season ranges from five to eight (data from 1985–1993).

Study site

The bluegill population is from Lake Opinicon, an 890-ha mesotrophic lake typical of those in southeastern Ontario (Keast 1978). It has a maximum depth of 11 m and an average depth of 4.5 m, and is usually covered with ice from December to April. Ice-out occurs in the spring as early as the first week in April and as late as the first few days in May. In 1994 ice-out occurred on approximately 21 April.

Shallow littoral areas are abundant and make up approximately 10% of the lake surface area (Curran et al. 1947). Lake Opinicon sustains a large natural population of bluegill sunfish and a total community of 18 species including 5 other species of centrarchids (Keast 1978). The reproductive biology of bluegill sunfish has been studied in the lake annually since the mid-1970s (e.g., Colgan et al. 1979; Gross 1980, 1982; Coleman et al. 1985; Claussen 1991; Gross 1991; Cargnelli and Gross[2]).

Reproductive activity surveys

Bluegill reproductive activity was studied in an area bounding 2 km of littoral zone along the northern shore of the lake. This zone, referred to as the fish reproduction study area (FRSA), includes a variety of habitat types characterizing the wide biological and physical diversity in which bluegill are known to spawn. The FRSA was monitored by snorkellers on a daily basis during the 1993 spawning season beginning several days before the onset of spawning activity and continuing for as long as males remained on nests. Thus, a complete record of all reproductive activity occurring within the study site during 1993 was obtained.

[2] L.M. Cargnelli and M.R. Gross. Fish energetics: larger individuals emerge from winter in better condition (bluegill sunfish: *Lepomis macrochirus*). Submitted for publication.

Dates of male colony formation, female spawning, egg hatch, and swim-up as well as the number of nests formed, the number of nests in which fish spawned, and the number of nests to produce free swimming fry were recorded for each colony that formed. Fry swim-up was not directly observed; instead, empty nests that on the previous day had contained parental males and larvae at the golden-eyed stage, the final larval stage in the nest, were taken as an indication that swim-up had occurred. Since surveys were performed in the mornings, the date of swim-up for each colony was assigned to the previous day, even though swim-up may have occurred earlier in the morning before our survey. Thus, the number of days from first to last swim-up in 1993 has an error of 1 day in either direction.

There were eight spawning bouts in 1993, with the first spawning occurring on 6 June (bout 1) and the final spawning on 13 July (bout 8). The first swim-up occurred on 14–15 June and the final swim-up on 17–18 July, a range of 32–34 days.

Estimates of egg number

The number of eggs within each nest on the day following spawning was estimated using a standard method developed in our laboratory. A diver visually examines the mass of eggs in a nest and assigns an egg score ranging from 0 to 5, with 0 indicating no eggs and each higher score representing increased numbers of eggs. Visual egg scores and the actual number of eggs in the nest are significantly correlated with little overlap between egg score categories (Claussen 1991). The number of eggs per egg score, determined by collecting and counting full clutches of eggs that had been scored, is as follows: score 1, 1–4900 eggs ($n = 9$ nests); score 2, 4600 – 29 000 ($n = 8$); score 3, 27 000 – 53 000 ($n = 7$); score 4, 49 000 – 87 000 ($n = 5$); score 5, 82 000 – 113 000 ($n = 3$) (data in Claussen 1991). Thus, although there is variability within each egg score, a reasonably accurate representation of differences among nests can be obtained.

Egg scores were estimated for all males that spawned within a colony and for all 106 colonies that formed within the FRSA in 1993. For some very large colonies ($n > 100$ nests), however, only a sample of nests including at least 50% of the individuals randomly distributed throughout the colony was scored. Egg scoring was tested for repeatability in two ways. First, all 49 nests in one colony were scored by two individuals and concordance was tested (Spearman rank correlation: $n = 49$, $r = 0.7255$, $p < 0.001$). Second, the average scores from all nests within five colonies scored by the two observers were compared (2.231 ± 0.787 (mean ± standard deviation) versus 2.359 ± 0.816; Mann–Whitney U test: df = 311, $Z = -1.67$, $p = 0.095$).

Estimates of fry production

We define production by the number of fry leaving the nest. There is a significant positive correlation between egg scores and the number of fry present in a nest on the final day of parental care (Spearman rank correlation: $n = 31$ nests, $r = 0.878$, $p = 0.0001$, data in Claussen 1991). Production from each colony that formed in 1993 was therefore calculated by multiplying the average egg score of a colony by the number of males in the colony that produced free-swimming fry. We used average egg scores instead of individual egg scores because we could not keep track of which individual nests in a colony were successful. The total production for each of the eight spawning bouts in 1993 was determined by summing the production estimates of each colony within each spawning bout.

Recruitment to age 1

Age-1 bluegill sunfish were captured using seine and minnow traps from within the FRSA on 24 May 1994. The total length (in millimetres) and wet weight (in grams) of each of the 400 fish were measured and all samples frozen. Otolith daily growth rings were used to age and obtain growth data on each fish. The presence of otolith daily growth rings has been verified in bluegill (Taubert and

Coble 1977) and in other centrarchids (Miller and Storck 1982; Taubert and Tranquilli 1982).

The saggital otoliths, the largest of the three pairs found in the inner ear, were removed from thawed fish using forceps and a dissecting microscope. Any soft tissue that adhered to the otolith was removed. The otoliths were then placed in 80% ethanol and the cleaning process was completed with fine forceps under a dissecting scope. The otoliths were allowed to air dry before their length was measured using a compound microscope and ocular micrometer (range: 1.6–2.1 mm). Otoliths were then mounted onto microscope slides.

The otoliths were embedded in Araldite GY-502 epoxy, which has been found to be the best medium for mounting otoliths because it is hard and clear and has a low refractive index (Cassleman and Barnes[3]). A thin layer of epoxy was placed on a microscope slide and the otoliths were positioned with the convex side up. After hardening for 24 h, the epoxy-embedded otoliths were ground and polished to thin the nucleus and increase optical resolution. Generally, otoliths smaller than 2 mm in length are thin enough that they need only be ground and polished on one side (Cassleman and Barnes[3]). The otoliths were ground lightly on a 600 grit disk and then more finely ground and polished with 12- and 0.3-μm aluminum oxide lapping film until the nucleus, the portion of the otolith produced during embryonic development (Taubert and Coble 1977), was exposed.

Daily rings were counted using a compound microscope at a magnification of 200–400×. The microscope was equipped with a camera lucida, which allowed the image to be projected onto a flat surface. This facilitated reading of the microzonation and allowed the otolith to be traced. Counts were made at the posterior end of the otolith as this is where optical microzonation is best resolved (Pannella 1974; Cassleman and Barnes[3]). Microstructure during the over-winter period could not be resolved because of the close proximity of the rings. Thus, counts were made from nucleus to annulus and represented the number of days from swim-up to the termination of growth in the fall.

Problems encountered while reading otoliths were similar to those described by Parrish et al. (1994). Cracks, accessory primordia, and loss of the nucleus or otolith margin made otolith analysis difficult, and uninterrupted viewing of the growth record was not always possible. In such cases, a single counting axis was not used; instead, multiple axes, which allowed rings to be read from nucleus to edge, were utilized. Where ring structure could not be discerned, the otolith was discarded. Counts were successfully made on 161 of 195 otoliths (83%).

Each individual's ring count was converted to the date of swim-up in the following manner. We expected a range of 32–34 days in the ring counts obtained from the 1994 yearlings because this was the number of days between the first and last swim-up observed in 1993. We assumed that the largest ring count corresponded to the first swim-up date, the smallest to the final swim-up date, and each count in between to the intervening dates. In this manner, each individual was assigned to one of the eight spawning bouts in 1993. Each individual was also assigned to an early-, middle-, or late-season trimester in 1993, each 11 days in length.

To estimate actual birth dates from our measures of the number of growth days, we assumed that growth in the fall ceased on the same day for each individual. This assumption seems generally reasonable from several lines of evidence. First, there is a strong relationship between feeding activity and temperature (e.g., Hathaway 1927; Marcus 1932; Gerking 1966; Keast 1977; Shuter and Post 1990) and between growth and temperature (e.g., Gerking 1966; Brett and Groves 1979; Peters 1983; Weatherley and Gill 1987; Shuter and Post 1990) in fish. The bluegill stops feeding with even a 0.5°C temperature drop. It is possible that all of the fish experience similar temperatures and thus respond similarly. Second, studies of seasonal growth cycles in bluegill (Gerking 1966; Keast 1995) suggest that even different age-classes stop growing at the same time in the fall. We expect much less variation within than among year-classes. Finally, and most importantly, the number of days of growth indicated by our otolith study corresponds exactly to the number of days predicted by the data collected from the reproductive activity surveys, a correspondence unlikely to occur by chance.

[3] J.M. Casselman and L.E. Barnes. Removal, storage, and preparation of fish otoliths for studies of microzonation: with special reference to young-of-the-year walleye. Presented at the Age and Growth Workshop – Otolith Method, Maple, Ont., 1987.

Fry size and growth

The average daily growth increments of yearlings were calculated as the total length of the fish divided by its age (millimetres per day). To examine if growth rate differed through the spawning season, regression analysis of yearling total length against yearling age was used. A separate regression was performed for early-, middle-, and late-season periods, and the homogeneity of the regression lines (slopes and intercepts) from each trimester was tested using an analysis of covariance. Significant differences in slopes among lines would indicate that growth rates varied at different times in the season. We used this technique to analyze trends in growth rather than simply analyzing the average daily growth increments over time because of the difficulties in dealing with spurious correlations (Jackson and Somers 1991).

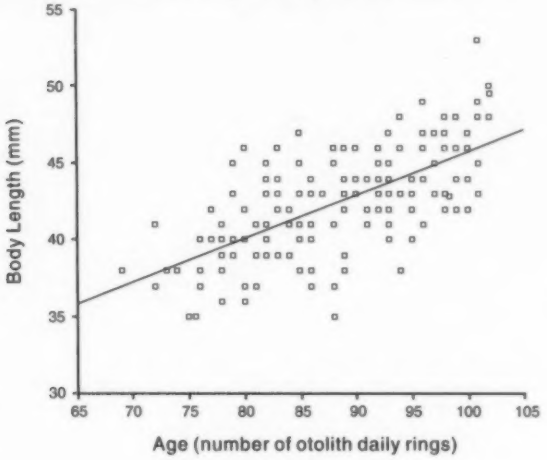
To quantify fry sizes among spawning bouts in 1993, samples of 10 fry were collected with a turkey baster from several nests from each spawning bout and preserved in 90% alcohol. The total length of each fry was measured using a dissecting microscope and ocular micrometer. Only fry that had reached the final golden-eyed stage, where the yolk sac is almost completely depleted and growth in the nest is complete, were used. Fry collected from the first and second bouts of spawning possessed visible yolk sacs and thus were discarded from the analysis.

Results

There was a 34-day range of ring counts made from yearling otoliths, from 69 to 102. This corresponds very closely to the 32–34 days expected on the basis of the first and last observed swim-up dates in 1993. Fish with the largest ring count were therefore assigned the date of 15 June, the first date on which fry were recruited. This implies that 1993 recruits stopped growing by 24 September 1993 (June 15 + 102 days), which is consistent with results of several studies of seasonal growth cycles in bluegill indicating that body growth ceases between mid-September and early October (Gerking 1966; Keast and Eadie 1984).

The 1993 recruits varied considerably in body size by the spring of 1994, ranging in size from 35 to 53 mm total length. These individuals were collected before significant spring growth had occurred, as evidenced by the small number of distinct postannulus growth rings. Thus, spring length is probably a good approximation of fall 1993 body size. Yearling body size was found to be highly correlated with the number of daily growth rings counted (Fig. 1). Older individuals, produced early in 1993, achieved a larger size at age 1 than those born later. Moreover, there was a progressive decrease in yearling body size with each successive spawning bout in 1993 (Fig. 2*a*; $df = 7$, $F = 20.06$, $p < 0.001$). These data are also summarized for early-, middle-, and late-born 1993 recruits (Fig. 3*a*; $df = 2$, $F = 48.98$, $p < 0.0001$). Recruits born early in

Fig. 1. Relationship between the number of otolith daily rings from nucleus to annulus in 1993 recruits and the fish's total body length in spring 1994 ($n = 161$, $r^2 = 0.451$, $p < 0.0001$). Larger ring numbers correspond to earlier birth dates in 1993.



1993 were 7.4% larger than midseason recruits and 13.6% larger than late-season recruits.

The average daily growth increment determined from the regression of total length plotted against ring number (Fig. 1) was 0.55 mm/day. However, growth increments varied among individuals from 0.40 to 0.57 mm/day. This variation was not time dependent as there was no significant difference in growth rates among early-, middle-, and late-season recruits on the basis of results from the analysis of covariance (Table 1). The interaction term in the first part of Table 1 indicates that regression slopes were equal across trimesters ($p = 0.677$), suggesting that all fry increased in size at the same rate through the growth season. The trimester term in the second part of Table 1 indicates that the y intercepts were significantly different ($p < 0.001$). This is expected, since early-season fry are larger at age 1 than middle- and late-season fry.

The body size of fry leaving the nest ranged from approximately 4.3 to 6.7 mm and was negatively correlated with spawn date ($n = 433$, $r^2 = 0.202$, $p < 0.001$). Thus, fry from earlier in the season were larger at swim-up than those from later. However, fry size did not make a statistically significant contribution to final recruit size as indicated by a multiple regression analysis with fall size as the dependent variable and spawning bout and fry size the independent variables (Table 2). Thus, the length of the 1993 growing season, as determined by otolith ring counts, appeared to be the most important factor in determining eventual body size in spring 1994.

Numbers of fry produced varied considerably throughout the 1993 breeding season, with the majority of fry being produced during the middle part of the season (Fig. 2*b*). The early season contributed a much smaller proportion of the total fry production than the middle or late season (Fig. 3*b*). The early parts of the season were only 37.7% as productive as the middle-season bouts and 46.3% as productive as the late-season bouts. However, the early season produced a relatively larger proportion of recruits into 1994 (Figs. 2*c*, 3*c*). If survival of

Fig. 2. Data summarized by 1993 spawning bout. (*a*) Total length (mm) (±1 SE) of yearlings in spring 1994 from each 1993 bout. Numbers represent sample sizes. (*b*) Proportion of the total fry produced in 1993 from each 1993 bout. Numbers represent actual proportions. (*c*) Proportion of recruits from each 1993 bout in spring 1994. Numbers represent actual proportions. (*d*) Relative proportional survivorship to spring 1994 for each 1993 bout, calculated by dividing the proportion of fry produced in 1993 (*b*) into the proportion of recruits in 1994 (*c*). The broken line represents a survival value of 1.0. A value above the line indicates an increase in representation; a value below indicates a decrease.

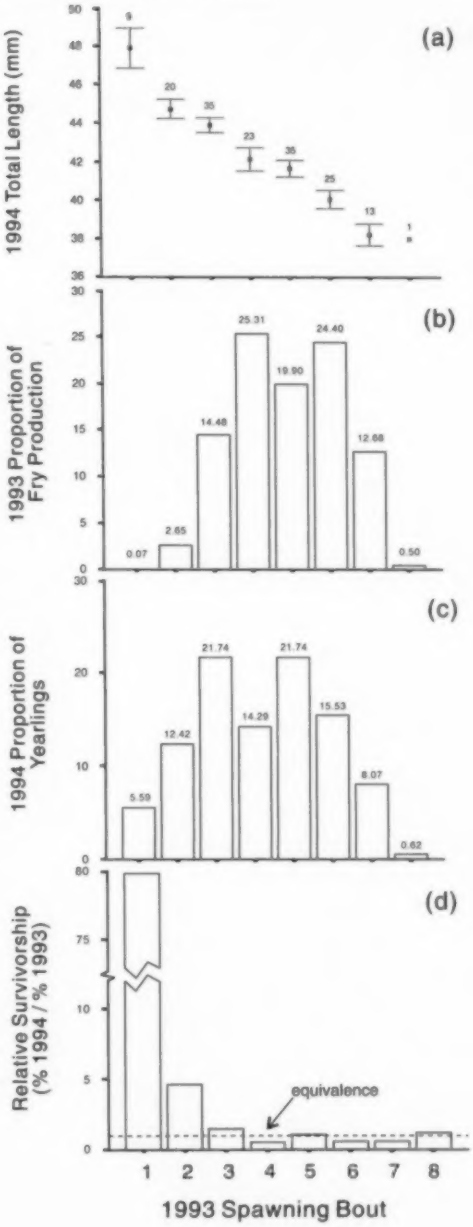
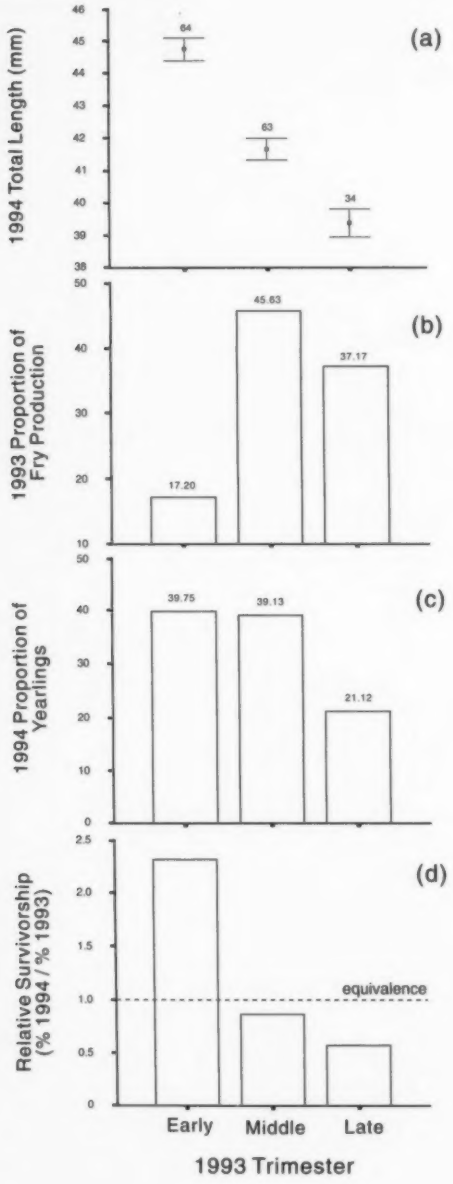


Fig. 3. Data summarized by 1993 trimester. (*a*) Total length (mm) (±1 SE) of yearlings in spring 1994 from each 1993 trimester. Numbers represent sample sizes. (*b*) Proportion of the total fry produced in 1993 from each 1993 trimester. Numbers represent actual proportions. (*c*) Proportion of recruits from each 1993 trimester in spring 1994. Numbers represent actual proportions. (*d*) Relative proportional survivorship to spring 1994 for each 1993 bout, calculated by dividing the proportion of fry produced in 1993 (*b*) into the proportion of recruits in 1994 (*c*). The broken line represents a survival value of 1.0. A value greater than 1.0 indicates an increase in representation; a value less than 1.0 indicates a decrease.



fry to age 1 were uniform across time, then we would expect the distribution of age-1 bluegill in 1994 to be the same as the distribution of fry in 1993. Chi-square tests indicated that the distribution of age-1 fish in 1994 was significantly different than that expected from 1993 fry production (by trimester: df = 2, $\chi^2 = 37.42$, $p < 0.0001$; by bout: df = 7, $\chi^2 = 484.83$, $p < 0.0001$).

The ratio of 1994 to 1993 proportions yields a measure of survival that can be compared across spawning bouts (Fig. 2*d*). For example, fry produced during the first bout of spawning in 1993 made up 0.07% of all 1993 fry, but made up 5.59% of recruits into 1994. This resulted in a relative proportional survivorship of 79.81 (5.59/0.07), indicating that the first bout produced about 80 times more recruits than expected on the basis of the 1993 numbers. The relative proportional survivorship tended to decrease with successive bouts, a trend that was more clear when the breeding season was divided into trimesters (Fig. 3*d*). Early-born individuals increased dramatically in representation (231% of 1993); middle-season individuals decreased slightly, and late-season individuals dropped sharply. Note that survivorship in the final bout (Fig. 2*d*) looks higher than might be expected. However, this may simply be an artifact of sampling bias as this was the only age-1 individual found to be from the eighth spawning bout.

Discussion

This study directly links the production of free-swimming fry from bluegill sunfish nests to recruitment into the age-1 year-class. Fry from the first trimester contributed substantially (40%) to year-class composition even though they represented a small proportion of the total fry production (17%), a 231% increase. Thus, early-season fry contributed disproportionately to the cohort. Moreover, the study shows that early-season fry obtain larger body sizes as 1 year olds, probably because of the longer growing season, which may account for their increased survivorship.

Past studies of survivorship to age 1 have not made the connection between survivorship and date of birth. We know that size-dependent processes can have considerable effects on the survivorship of young-of-the-year individuals. A number of studies have demonstrated that predation can be size selective, affecting more intensely the smaller individuals in a population (Post and Prankevicius 1987; Post and Evans 1989*a*; but for an exception see Litvak and Leggett 1992). Other studies indicate that larger individuals have a greater probability of surviving winter starvation periods (Oliver et al. 1979; Toneys and Coble 1979; Shuter et al. 1980; Post and Evans 1989*b*; Smith and Griffith 1994). On the basis of these results, we expect the larger individuals in a population or cohort to be favored and to show the highest survivorship. But who are the larger individuals in a cohort and from where do they come? This issue has not been previously addressed, and no study has demonstrated the link between fry production and actual recruitment to age 1. Thus, the results of this study advance our understanding of the relationship between recruitment and size-dependent processes.

The data clearly show that early-emerging fry attain the largest body size by winter. The most obvious explanation for this is that early-season young of the year have available to them a longer growing season. The earliest born fry in 1993

Table 1. Results from an analysis of covariance comparing changes in body size with age of early-, middle-, and late-season recruits.

	SS	df	MS	*F*	*p*
Test for differences in slopes					
Error	812.93	129	6.30		
Age	171.67	10	17.17	2.72	0.005
Trimester	628.67	2	314.33	49.88	<0.001
Trimester × age	98.47	19	5.18	0.82	0.677
Test for differences in intercepts					
Error	994.47	157	6.33		
Age	124.36	1	124.36	19.63	<0.001
Trimester	810.03	2	405.02	63.94	<0.001

Note: Regression lines from each trimester are tested for significant differences in slopes, and intercepts. SS, sum of squares; df, degrees of freedom; MS, mean square.

Table 2. Results of a multiple regression analysis testing the relative contributions of birth date (spawning bout) and fry size to the body size attained by bluegill sunfish recruits ($r^2 = 0.983$, $F = 56.373$, $p = 0.0087$).

Variable	β	*t*	*p*
Spawning bout	−1.055	−9.214	0.0116
Fry size at swim-up	−0.118	−1.033	0.4103
Constant		10.622	0.0087

had 48% more growing days than the latest born. Moreover, there is a progressive decline in fall body size with each successive bout of spawning. Therefore, the more time they have to grow, the larger they get. Alternative explanations of the trend seem less plausible. First, if early-season fry have greater food resources available to them, then larger daily growth increments should be associated with early-born individuals. However, growth rates were highly variable and showed no relationship with birth date. Second, our data indicate that fry size at swim-up decreases slightly through the spawning season. However, relative to the length of the growing season, fry size does not make a statistically significant contribution to the variation in age-1 body size. Third, early- and late-season fry obtained similar size at age.

Early-emerging fry also have the highest survival to age 1. There are three potential hypotheses that can explain this result. First, the larger fall body size attained by early-season fry may result in greater over-winter survival. Since body size is allometrically related to metabolic rate and energy reserves (Brett and Groves 1979; Shuter and Post 1990), and bluegill do not feed during the winter (Keast 1968; Keast 1977), larger individuals should have the highest probability of surviving to spring (Cargnelli and Gross, see footnote 2). Our data are consistent with this, as body size and survival are highly correlated.

A second explanation to consider is size-selective predation during the growing season. Post and Prankevicius (1987) found that growth rates and the composition of the predator population determine whether predation has a significant size-dependent effect. Early-emerging bluegill fry did not have the advantage of faster growth rates, and so all young of the year spent similar amounts of time within vulnerable size-classes (Werner and Gilliam 1984). Predation on age-0 bluegill in Lake Opinicon comes from a number of sources (Keast 1985): (*i*) all age-classes of bass, *Micropterus dolomieu* and *Micropterus salmoides*; (*ii*) older age-classes of yellow perch, *Perca flavescens*; and (*iii*) all age-classes of northern pike, *Esox lucius*. These predators are present throughout the growing season and are probably not gape limited. Thus, all young of the year were probably exposed to similar predation pressures. This does not, however, fully exclude the possibility of size-selective predation during the growing season, since other factors such as predator-avoidance capability may be size dependent.

Third, middle- and late-season fry may experience greater mortality relative to early-season fry as a result of food limitation. However, this is unlikely because middle- and late-season fry grew at approximately the same rate as early-emerging fry and attained a size at age similar to that of the early-season fry.

The phenomenon of size-dependent over-winter mortality is receiving increasing attention, in both experimental (Oliver et al. 1979; Toneys and Coble 1979; Post and Evans 1989*b*; Smith and Griffith 1994) and descriptive studies (Toneys and Coble 1979; Shuter et al. 1980; Post and Evans 1989*b*). Our results correlate survivorship with actual spawning dates, showing that larger individuals with higher survivorship are coming from the early parts of the season. This suggests that size-dependent over-winter mortality can be an important process operating within a cohort and indicates the value of early season spawning to the year-class and population. If the size and survivorship advantages of early season fry were to accumulate each year to adulthood (Baylis et al. 1993), then the value of early spawning would be even greater than indicated by our results. Yearlings in our study varied in size by 18 mm, or 50%, after less than 1 year. Parental males of age 8, the most common breeding age-class, vary in size by 59 mm, or 38% ($n = 197$), a result, at least in part, of this variation in yearling size. Larger males have significantly higher breeding success (Gross 1980), and thus early-season fry contribute the most to the population in terms of numbers, and probably also as the largest, most valuable fish in the population.

Our study also suggests that variation in the length and quality of the growing season will strongly affect year-class strength. Small changes in the growing season, either by a delayed spring or early fall, may prevent individuals from attaining a minimum body size required to survive the winter. Thus, in a year in which the spring is cold and spawning is delayed, the average fall size attained by young of the year would decrease, resulting in increased total over-winter mortality and a weak year-class. Conversely, in an early season the average fall body size is increased and the total over-winter mortality should decrease. Although very simplistic, these scenarios show the importance of understanding the temporal dimension within a cohort to understand year-class dynamics.

Finally, the results of this study hold interesting implications for the breeding structure of bluegill sunfish. First, the higher value of early-season fry will favor adult males and females that spawn early. However, there are costs associated with early nesting for males and only individuals that can afford to pay these costs will nest early (L.M. Cargnelli and M.R. Gross, University of Toronto, unpublished data). As

well, differences in the value of offspring at various times in the season (from Fig. 3*d*: 2.3 versus 0.9 versus 0.6) will have important impacts on the investment decisions of parental males (Coleman et al. 1985). For example, early-spawning parental males should invest more effort in a clutch of the same size as one spawned later in the season, all else being equal. Third, the size and survivorship advantages of early fry may accumulate through to maturity at age 7 or 8 years for parental males and age 5 or 6 years for females (Gross 1980). Since only the largest and fastest growing males in the parental life history mature at 7 years (Gross and Charnov 1980), this would save 1 year of mortality and further ensures the breeding contribution of early-season fry. Finally, the relationship between seasonal birth date and the relative production of males to the alternative life histories of cuckolder and parental males (Gross 1982, 1991) remains to be explored. Males maturing as cuckolders do so at age 2 and sneak fertilizations from the nests of parental males. If body size is more important to the cuckolder than parental life history, then cuckolder males may constitute a greater proportion of early-season recruits.

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References

Baylis, J.R., Wiegmann, D.D., and Hoff, M.H. 1993. Alternating life histories of smallmouth bass. Trans. Am. Fish. Soc. **122**: 500–510.

Brett, J.R., and Groves, T.D.D. 1979. Physiological energetics. *In* Fish physiology. Vol. 10. *Edited by* W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. pp. 279–352.

Claussen, J.E. 1991. Annual variation in the reproductive activity of a bluegill population: effect of clutch size and temperature. M.Sc. thesis, University of Toronto, Toronto, Ont.

Coleman, R.M., Gross, M.R., and Sargent, R.C. 1985. Parental investment decision rules: a test in bluegill sunfish. Behav. Ecol. Sociobiol. **18**: 59–66.

Colgan, P.W., Nowell, W.A., Gross, M.R., and Grant, J.W.A. 1979. Aggressive habituation and rim circling in the social organization of bluegill sunfish (*Lepomis macrochirus*). Environ. Biol. Fishes, **4**: 29–36.

Curran, H.W., Bardach, J., Bowman, R.I., and Lawler, H.G. 1947. A biological survey of Lake Opinicon: progress report from the Queen's University Biological Station. Queen's Unversity, Kingston, Ont.

Fogarty, M.J., Sissenwine, M.P., and Cohen, E.B. 1991. Recruitment variability and the dynamics of exploited marine populations. Trends Ecol. Evol. **6**: 241–246.

Hathaway, E.S. 1927. The relation of temperature to the quantity of food consumed by fishes. Ecology, **8**: 428–434.

Gerking, S.D. 1966. Annual growth cycle, growth potential, and growth compensation in the bluegill sunfish in northern Indiana lakes. J. Fish. Res. Board Can. **23**: 1923–1956

Gross, M.R. 1980. Sexual selection and the evolution of reproductive strategies in sunfishes (*Lepomis*: Centrarchidae). Ph.D. thesis, University of Utah, Salt Lake City, Utah.

Gross, M.R. 1982. Sneakers, satellites, and parentals: polymorphic mating strategies in North American sunfishes. Z. Tierpsychol. **60**: 1–26.

Gross, M.R. 1991. Evolution of alternative reproductive strategies: frequency-dependent sexual selection in male bluegill sunfish. Philos. Trans. R. Soc. Lond. B Biol. Sci. **332**: 59–66.

Gross, M.R., and Charnov, E.L. 1980. Alternative male life histories in bluegill sunfish. Proc. Natl. Acad. Sci. U.S.A. **77**: 6937–6940.

Jackson, D.A., and Somers, K.M. 1991. The spectre of 'spurious' correlations. Oecologia, **86**: 147–151.

Keast, A. 1968. Feeding of some Great Lakes fishes at low temperatures. J. Fish. Res. Board Can. **25**: 1199–1218.

Keast, A. 1977. Mechanisms expanding niche width and minimizing intraspecific competition between two centrarchid fishes. *In* Evolutionary biology. Vol. 10. *Edited by* M.K. Hetcht, W.C. Steere, and B. Wallace. Plenum Press, New York. pp. 333–395.

Keast, A. 1978. Trophic and spatial interrelationships in the fish species of an Ontario temperate lake. Environ. Biol. Fishes, **3**: 7–31.

Keast, A. 1985. The piscivore feeding guild of fishes in small freshwater ecosystems. Environ. Biol. Fishes, **12**: 119–129.

Keast, A. 1995. Long-term studies of northern temperate freshwater fish communities. *In* Long-term ecological studies. *Edited by* M.L. Cody and J.A. Smallwood. Academic Press, New York. In press.

Keast, A., and Eadie, J. 1984. Growth in the first summer of life: a comparison of nine co-occurring fish species. Can. J. Zool. **63**: 1242–1250.

Litvak, M.K., and Leggett, W.C. 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. Mar. Ecol. Prog. Ser. **81**: 13–24.

Marcus, H.C. 1932. The extent to which temperature changes influence food consumption in large-mouth bass *(Huro floridana)*. Trans. Am. Fish. Soc. **62**: 202–210.

Miller, S.J., and Storck, T. 1982. Daily growth rings in otolith of young-of-the-year largemouth bass. Trans. Am. Fish. Soc. **111**: 527–530.

Miller, T.J., Crowder, L.B., Rice, J.A., and Marshall, E.A. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. **45**: 1657–1670.

Oliver, J.D., Holeton, G.F., and Chua, K.E. 1979. Overwinter mortality of fingerling smallmouth bass in relation to size, relative energy stores, and environmental temperature. Trans. Am. Fish. Soc. **108**: 130–136.

Pannella, G. 1974. Otolith growth patterns: an aid in age determination in temperate and tropical fishes. *In* Ageing of fish. *Edited by* T.B. Bagenal. The Gresham Press, Old Woking, Surrey, U.K. pp. 28–39.

Parrish, D.L.,Vondracek, B., and Eckmayer, W.J. 1994. Accuracy and precision of daily age estimates for walleyes from Ohio hatcheries and Lake Erie. Trans. Am. Fish. Soc. **123**: 601–605.

Peters, R.H. 1983. The ecological implications of body size. Cambridge University Press, New York.

Post, J.R., and Evans, D.O. 1989*a*. Experimental evidence of size-dependent predation mortality in juvenile yellow perch. Can. J. Zool. **67**: 521–523.

Post, J.R., and Evans, D.O. 1989*b*. Size-dependent overwinter mortality of young-of-the-year yellow perch (*Perca flavescens*): laboratory, in situ enclosure, and field experiments. Can. J. Fish. Aquat. Sci. **46**: 1958–1968.

Post, J.R., and Prankevicius, A.B. 1987. Size-selective mortality in young-of-the-year yellow perch (*Perca flavescens*): evidence from otolith microstructure. Can. J. Fish. Aquat. Sci. **44**: 1840–1847.

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184. pp. 1–966.

Shuter, B.J., and Post, J.R. 1990. Climate, population variability, and the zoogeography of temperate fishes. Trans. Am. Fish. Soc. **119**: 314–336.

Shuter, B.J., MacLean, J.A., Fry, F.E.J., and Regier, H.A. 1980. Stochastic simulation of temperature effects on first-year survival of smallmouth bass. Trans. Am. Fish. Soc. **109**: 1–34.

Smith, R.W., and Griffith, J.S. 1994. Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. Trans. Am. Fish. Soc. **123**: 747–756.

Taubert, B.D., and Coble, D.W. 1977. Daily growth rings in otoliths of three species of *Lepomis* and *Tilapia mossambica*. J. Fish. Res. Board Can. **34**: 332–340.

Taubert, B.D., and Tranquilli, J.A. 1982. Verification of the formation of annuli in otoliths of largemouth bass. Trans. Am. Fish. Soc. **111**: 531–534.

Toneys, M.L., and Coble, D.W. 1979. Size-related, first winter mortality of freshwater fishes. Trans. Am. Fish. Soc. **108**: 415–419.

Weatherley, A.H., and Gill, H.S. 1987. The biology of fish growth. Academic Press, London.

Werner, E.E., and Gilliam, J.F. 1984. The ontogenetic niche and species interactions in size-structured populations. Annu. Rev. Ecol. Syst. **15**: 393–425.

Coronary vascular smooth muscle responses to swimming challenges in juvenile salmonid fish

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Abstract: Using [^{3}H]thymidine to measure the mitotic activity of medial vascular smooth muscle (VSM) from salmonid coronary arteries, we tested the hypothesis that periodic anaerobic swimming induces greater VSM mitotic activity than sustained aerobic swimming. Juvenile chinook salmon were swum either at a submaximal speed of 1.5 body lengths/s for 8 months alone, or at a combination of forced swims to exhaustion (critical swimming speed) on alternate days in addition to a continuous low speed of 0.5 body lengths/s for 3 months. Fish swum at a continuous low speed (0.5 body lengths/s) for either 3 or 8 months acted as controls. Rainbow trout were forced to burst swim to exhaustion twice daily for 3 days. Mitotic activity in coronary arterial explants was measured at the end of each of the regimes using [^{3}H]thymidine. Compared with controls, the combination of exhaustive swimming on alternate days and continuous submaximal swimming at 1.5 body lengths/s stimulated coronary VSM mitosis. Neither submaximal swimming alone nor burst swimming stimulated coronary VSM mitosis. We conclude that some but not all forms of periodic anaerobic swimming induce coronary VSM mitosis, with the duration and number of swimming events likely being important considerations.

Résumé : Utilisant la [^{3}H]thymidine pour mesurer l'activité mitotique du muscle lisse vasculaire médial provenant des artères coronaires de salmonidés, nous avons vérifié l'hypothèse selon laquelle la nage périodique en conditions anaérobies induit une plus grande activité mitotique du muscle lisse vasculaire que la nage aérobie soutenue. On a fait nager des saumons quinnats juvéniles soit à une vitesse sous-maximale de 1,5 longueur corporelle/s pendant 8 mois, soit selon une combinaison de nage forcée jusqu'à l'épuisement (vitesse de nage critique) 1 jour sur 2 et de nage à une faible vitesse continue de 0,5 longueur corporelle/s pendant 3 mois. Des poissons nageant à une faible vitesse continue (0,5 longueur corporelle/s) pendant 3 ou 8 mois servaient de témoins. On a forcé des truites arc-en-ciel à nager de façon intermittente jusqu'à épuisement deux fois par jour pendant 3 mois. On a mesuré à l'aide de la [^{3}H]thymidine l'activité mitotique d'explants de l'artère coronarienne à la fin de chacune des expériences. Par rapport aux témoins, la combinaison de la nage forcée 1 jour sur 2 et de la nage à une vitesse sous-maximale de 1,5 longueur corporelle/s stimulait la mitose du muscle lisse vasculaire. Ni la nage à une vitesse sous-maximale seule ni la nage intermittente ne stimulaient la mitose du muscle lisse vasculaire. Nous concluons que certains types de nage anaérobie périodique, mais non pas tous, induisent la mitose du muscle lisse vasculaire de l'artère coronaire, et que la durée et le nombre de périodes de nage sont probablement des facteurs importants de ce phénomène.
[Traduit par la Rédaction]

Introduction

Perhaps the best-studied morphological reconstructions of blood vessels in response to an environmental stress are the changes associated with mammalian arteriosclerosis. Migratory salmonids also develop coronary arteriosclerotic lesions (Robertson et al. 1961; see review in Farrell and Jones 1992), but in contrast to the situation in mammals the factors that initiate these changes are not known. Because vascular smooth muscle (VSM) is the major cell type in fish coronary lesions (Moore et al. 1976*a*, 1976*b*), initiating events for lesion formation in salmonids probably involve migration and proliferation of medial VSM, as they do in mammals (Ross and Glomset 1973).

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A technique to study the mitotic activity of medial VSM from salmonid coronary arteries has recently been developed (Gong and Farrell 1995). We used this technique to examine the consequences of three different types of swimming regimes on coronary arterial VSM mitosis. The working hypothesis is that periodic anaerobic swimming induces greater VSM activity than sustained aerobic swimming. This hypothesis acknowledges the importance of vascular injury in the etiology of lesions in mammals, as well as the suggestion that coronary lesions in salmonids are primarily initiated as a result of general mechanical injury to the coronary arterial wall that results from swimming involving anaerobic activities, e.g., avoiding predation and chasing prey (Saunders et al. 1992). Cumulative injury to the coronary artery would then lead to the severe coronary lesion formation observed in mature, migratory salmonids.

Materials and methods

All species of salmonids examined to date develop lesions in their coronary arteries, even though the severity of coronary lesions may differ somewhat among species (Farrell et al. 1990). These experiments involved juvenile chinook salmon *(Oncorhynchus tshawytscha)* (55–95 g) raised by the Department of Fisheries and Oceans

Table 1. Effect of training regimes on [^{3}H]thymidine incorporation into explants of coronary arterial smooth muscle of chinook salmon.

Fish group	n	Fish swimming speed (bl/s)	Duration (months)	[^{3}H]Thymidine (dpm/μg DNA)
Control	16	0.5	3–8	1435±242
Submaximal aerobic swimming	8	1.5	8	2116±326
Maximal aerobic swimming (U_{crit})	8	up to 2.8–2.9	3 (on alternate days)	3954±329*

Note: Two control groups were tested, one lasting 3 months, the other 8 months. There was no significant difference in [^{3}H]thymidine incorporation between the two groups and the data were pooled.

*Significantly different ($p < 0.05$) from control.

Canada as part of a much larger study (see Keissling et al. 1994), and rainbow trout (*Oncorhynchus mykiss*) (600–700 g) obtained from West Creek Trout Farm, Abbotsford, British Columbia. Chinook salmon were placed into 4000-L circular, outdoor, fiberglass tanks. Each tank was fitted with an inner fiberglass hoop, creating a circular swimming channel 45 cm wide and 55 cm deep. Aeration of the seawater was provided by Millipore tubing connected to compressed air. Water flow rates, temperatures, salinities, and dissolved oxygen concentrations during the experiment were in the range of 25–40 L/min, 8.2–12.3°C, 24–29‰, and 11–14 ppm, respectively. Rainbow trout were held at Simon Fraser University in 2000-L circular, outdoor, fiberglass tanks supplied with dechlorinated municipal water. The water temperature varied seasonally and was about 11–13°C during the experiments in October and November. During the period of holding, fish were fed daily to satiation with a commercial pellet diet (Oregon Moist).

Exercise regimes

One experiment examined the effect of submaximal continuous swimming for 8 months. A second experiment examined the effect of swimming to the maximal prolonged swimming speed (critical swimming speed; U_{crit}) on alternate days for 3 months. A third experiment examined the effect of brief bouts of burst swimming to exhaustion over 3 days.

Chinook salmon were exposed to either continuous low-speed swimming at 0.5 body length/s (bl/s), or continuous submaximal aerobic swimming (1.5 bl/s) for 8 months. The former acted as the control group. Water velocities in different parts of the circular swimming channel were measured in each tank every 2nd day using a flow meter (Braystoke BFM002, Valeport Marine Scientific, Devon, U.K.). No differences existed between the top and bottom of the water column. The velocity of 1.5 bl/s is around 50–60% of the maximal prolonged swimming speed; cardiac output would be elevated at this speed but not necessarily mean arterial blood pressure (Thorarensen 1994).

Another group of chinook salmon were exposed to either continuous low-speed swimming (0.5 bl/s) or continuous low-speed swimming (0.5 bl/s) plus swimming to their U_{crit} (2.8–2.9 bl/s) every 2nd day for 3 months. Again, the fish swimming continuously at low speed acted as the control group. U_{crit} was achieved by means of stepwise increases in the water velocity until fish became exhausted. Typically the fish swam for 1–3 h before becoming exhausted. Eight fish were sampled at the end of each of the training regimens. Kiceniuk and Jones (1977), for rainbow trout, and Thorarensen (1994), for chinook salmon, found that cardiac output and aortic blood pressure increased markedly as fish approached their maximal prolonged swimming speed. Thus, a likely cardiovascular event for chinook salmon swimming to U_{crit} was an elevated ventral aortic blood pressure and cardiac output, especially compared with fish swimming at 1.5 bl/s.

To study the effect of burst swimming over a short duration (3 days), eight rainbow trout were encouraged, by dipping a stick into the water, to swim vigorously in their circular tank until they were exhausted. The exercise regime was implemented twice daily for 3 days. Coronary arterial explants were removed for in vitro culture after the 3rd day. Eight undisturbed, freely swimming fish were used as a control group. Burst swimming typically exhausts fish within 10 min. There is an initial bradycardia and decreased cardiac output (Stevens et al. 1972; Farrell 1982), but with time and when the tail beats become less forceful, bradycardia gives way to tachycardia, and then cardiac output, catecholamine levels, and arterial blood pressure all increase.

Coronary artery explant

The method of in vitro [^{3}H]thymidine incorporation during the S phase of cell division followed that fully described by Gong and Farrell (1995) for fish coronary arteries. In brief, fish were killed by a sharp blow on the head. A long, straight portion of the coronary artery, consisting mainly of medial vascular smooth muscle and intimal tissue, was removed and was maintained in ice-chilled Hanks balanced salts (Sigma Chemical Co.) culture medium that had been modified by adding buffers (0.336 g/L $NaHCO_3$ and 4.588 g/L *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid) and the antibiotic gentamycin (0.065 g/L). During the 13-h incubation period at 15°C and with an atmosphere of 0.5% CO_2 – 99.5% air, the vessel segments were incubated at pH 7.6 ± 0.1 in 0.35 mL of culture medium using a 96-well culture plate (Corning Glass Works) containing 4 μL of [^{3}H]thymidine (40 MBq/mL, Amersham). At the end of the incubation period the coronary tissue was homogenized for DNA analysis using a fluorescent probe and a Perkin-Elmer LS50 fluorescence spectrophotometer linked to an IBM computer. The typical measurement range was 100–500 ng of DNA. After the DNA determination, radioactivity was counted for at least 5 min in a Beckman liquid scintillation counter (Beckman LS 3801). Counts were typically 500–800 dpm. The incorporation of [^{3}H]thymidine was expressed as disintegrations per minute (dpm) per microgram of DNA. Statistical comparisons were performed using Student's t test and a level of $p < 0.05$ was taken as significant. Results are expressed as the mean ± standard error of the mean (SEM).

Results

The [^{3}H]thymidine incorporation into coronary explants was not significantly different for the two control groups of chinook salmon that swam continuously at 0.5 bl/s for either 3 or 8 months. Therefore, these two control data sets were pooled for presentation in Table 1. The environmental stress of repeated U_{crit} swimming caused medial VSM proliferation. [^{3}H]Thymidine incorporation into coronary explants increased significantly ($p < 0.05$) after 3 months of swimming to U_{crit} on alternate days (Table 1). These fish would have swum to exhaustion approximately 45 times over the 3-month period, covering a total distance of about 442 km. In contrast, submaximal aerobic swimming for 8 months had no significant effect on VSM mitosis compared with the control group

(Table 1). With submaximal aerobic swimming alone, the fish would have covered a total distance of 3766 km over the 8-month period, but would not have been exhausted by the water current.

There was no significant difference ($p < 0.05$) in [^{3}H]thymidine incorporation into the coronary arterial smooth muscle of rainbow trout as a result of enforced burst swimming daily for 3 days (controls, 2953 ± 518; experimental, 1893 ± 393 dpm [^{3}H]thymidine/μg DNA; $n = 8$). These fish would have been exhausted 6 times, with a total swimming time of less than 60 min.

Discussion

Saunders et al. (1992) suggested that the development of coronary arterial lesions in salmonids may be linked to environmental stresses. The present study provides the first indirect support for such a suggestion. The stress of certain types of swimming stimulated medial VSM proliferation in coronary artery explants. Factors that cause medial VSM proliferation are likely to cause intimal VSM proliferation as well. The stimulus for VSM mitosis was related to repeated swimming to fatigue rather than long-term, submaximal endurance swimming (1.5 bl/s), which was not different from swimming at 0.5 bl/s in terms of VSM mitosis. Coronary VSM mitosis was stimulated after swimming to fatigue around 45 times over 90 days rather than after 6 brief burst swimming episodes over 3 days. Three days should have been ample time for a mitogenic response to develop, since peak mitotic activity occurred only 48 h after a single, gentle abrasion of the coronary artery to injure the vascular endothelium (Gong and Farrell 1995). A similar rapid VSM mitotic response to coronary endothelial injury also occurs in mammals. For example, Capron and Bruneval (1989) observed that the vascular media increased incorporation of [^{3}H]thymidine 2 days after injury with a balloon catheter. Further experiments are needed to explain why, of the two forms of periodic burst exercise that were tested, only one exerted a mitogenic effect on coronary VSM. It could be that more than six anaerobic swimming events must accumulate for the mitogenic stimulus. Alternatively, factors associated with sustained swimming at speeds greater than 1.5 bl/s (other than briefly in a burst mode) may confer important mitogenic stimuli for coronary VSM. Experiments are also needed to confirm that lesions accumulate as a result of increased mitogenic activity of coronary medial VSM.

Although we clearly show a linkage between a particular swimming challenge and increased coronary VSM mitotic activity, there are many possible interpretations for why this occurs. Given that direct mechanical abrasion of the coronary artery in vivo leads to a similar level of mitotic activity in coronary explants (Gong and Farrell 1995), it is tempting to speculate that injury to the coronary vascular wall is an important stimulus during periodic anaerobic swimming. Saunders et al. (1992) proposed that injury to the coronary arterial vascular wall may occur when excessive increases in ventral aortic blood pressure distend the ventricular outflow tract (bulbus arteriosus and ventral aorta) and cause stretching of the coronary artery. Such a possibility is now worth testing, especially given the fact that the endothelium of the trout coronary artery is quite easily removed or injured by mechanical disturbances (Small et al. 1990; Sverdrup et al. 1994) and that blood pressure will be elevated during anaerobic swimming. Furthermore, increased ventral aortic pressure in combination with increased cardiac output may be the critical hemodynamic events linked to coronary VSM mitosis given that both are substantially elevated at U_{crit}. Perhaps experiments involving ventral aortic banding in swimming fish would be fruitful in this regard. In the interim, it would be unwise to eliminate factors other than hemodynamic stimuli (e.g., catecholamines autooxidized to oxygen free radicals; Rump and Klaus 1994) as being the proximate mitogenic stimulus. Therefore, the possibility that swimming causes mitogenic stimuli for other fish tissues is an area worth exploring. It is likely that the technique used here could be easily adapted for other fish tissues.

In conclusion, one type of stressful swimming led to a significant mitogenic stimulation of coronary medial VSM. The finding of increased mitogenic activity after 3 months of repeated swimming to U_{crit} in chinook salmon provides indirect support for the idea that environmental stresses could initiate coronary arteriosclerosis in salmonids. Long-term experiments to specifically look for lesion formation in response to environmental stress can now be better designed.

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References

Capron, L., and Bruneval, P. 1989. Influence of applied stress on mitotic response of arteries to injury with a balloon catheter: quantitative study in rat thoracic aorta. Cardiovasc. Res. **23**: 941–948.

Farrell, A.P. 1982. Cardiovascular changes in the unanaesthetized lingcod (*Ophiodon elongatus*) during short-term, progressive hypoxia and spontaneous activity. Can. J. Zool. **60**: 933–941.

Farrell, A.P., and Jones, D.R. 1992. The heart. *In* Fish physiology. *Edited by* W.S. Hoar, D.J. Randall, and A.P. Farrell. Academic Press, New York.

Farrell, A.P., Johanson, J.A., and Saunders, R.L. 1990. Coronary lesions in Pacific salmonids. J. Fish Dis. **13**: 97–100.

Gong, B.Q., and Farrell, A.P. 1995. A method of culturing coronary artery explants for measuring vascular smooth muscle proliferation in rainbow trout. Can. J. Zool. **73**: 623–631.

Kiceniuk, J.W., and Jones, D.K. 1977. The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. J. Exp. Biol. **69**: 247–260.

Kiessling, A., Higgs, D.A., Dosanjh, B.S., and Eales, J.G. 1994. Influence of sustained exercise at two ration levels on growth and thyroid function of all female chinook salmon (*Oncorhynchus tshawytscha*) in seawater. Can. J. Fish. Aquat. Sci. **51**: 1975–1984.

Moore, J.F., Mayr, W., and Houghie, C. 1976*a*. Ultrastructure of coronary arterial changes in spawning Pacific salmon (genus *Oncorhynchus*) and steelhead trout (*Salmo gairdneri*). J. Comp. Pathol. **86**: 259–267.

Moore, J.F., Mayr, W., and Houghie, C. 1976*b*. Number, location and severity of coronary arterial changes in steelhead trout, *Salmo gairdneri*. Atherosclerosis, **24**: 381–386.

Robertson, V.H., Wexler, B.C., and Miller, B.F. 1961. Degenerative

changes in the cardiovascular system of the spawning Pacific salmon (*Oncorhynchus tshawytscha*). Circ. Res. **9**: 826–834.

Ross, R., and Glomset, J.A. 1973. Atherosclerosis and the arterial smooth muscle cell: proliferation of smooth muscle is a key event in the genesis of atherosclerosis. Science (Washington, D.C.), **180**: 1332–1339.

Rump, A.F.E., and Klaus, W. 1994. Evidence for norepinephrine cardiotoxicity mediated by superoxide anion radicals in isolated rabbit hearts. Naunyn-Schmiedebergs Arch. Pharmacol. **349**: 295–300.

Saunders, R.L., Farrell, A.P., and Knox, D.E. 1992. Progression of coronary arterial lesions in Atlantic salmon as a function of growth rate. Can. J. Fish. Aquat. Sci. **49**: 878–884.

Small, S.A., MacDonald, C., and Farrell, A.P. 1990. Vascular reactivity of the coronary artery in rainbow trout (*Oncorhynchus mykiss*). Am. J. Physiol. **258**: R1402–R1410.

Stevens, E.D., Bennion, G.R., Randall, D.J., and Shelton, G. 1972. Factors affecting arterial blood pressures and blood flow from the heart in intact, unrestrained lingcod, *Ophiodon elongatus*. Comp. Biochem. Physiol. **43**: 681–695.

Sverdrup, A., Kjellsby, E., Kruger, P.G., Floysand, R., Knudsen, F.R., Enger, P.S., Serck-Hanssen, G., and Helle, K.B. 1994. Effects of experimental seismic shock on vasoactivity of arteries, integrity of the vascular endothelium and on primary stress hormones of the Atlantic salmon. J. Fish Biol. **45**: 973–995.

Thorarensen, H. 1994. Gastrointestinal blood flow in chinook salmon (*Oncorhynchus tshawytscha*). Ph.D. thesis, Simon Fraser University, Burnaby, B.C.

Identification of annually produced opaque bands in the shell of the freshwater mussel *Elliptio complanata* using the seasonal cycle of $\delta^{18}O$

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Abstract: Three methods of aging *Elliptio complanata* were compared: (*i*) counting the number of opaque bands seen on the exterior of the shell using transmitted light, (*ii*) counting the number of opaque bands observed in polished longitudinal sections of shell, and (*iii*) counting the number of opaque bands identified as annual on the basis of their $\delta^{18}O$ concentration. On average, the number of opaque bands counted in the shell interior was greater than the number of opaque bands counted on the shell exterior. The number of annually produced opaque bands, distinguished from disturbance bands by their position within the seasonal cycle of $\delta^{18}O$ in the shell, agreed with the number of bands counted in polished interior sections. Opaque bands caused by nonannual events were either not continuous from the growing edge to the umbo, or not continuous until they merged into and with other bands near the pallial line. The counting of the internal opaque bands, using the procedures described in this study, provided the best estimate of the true age of *Elliptio complanata*.

Résumé : On a comparé trois méthodes de détermination de l'âge de *Elliptio complanata*, soit (*i*) le compte des bandes opaques à l'extérieur de la coquille observées en lumière transmise, (*ii*) le compte des bandes opaques observées dans des sections longitudinales polies de la coquille et (*iii*) le compte des bandes opaques identifiées comme étant annuelles d'après leur concentration de $\delta^{18}O$. En moyenne, le nombre de bandes opaques comptées à l'intérieur de la coquille était supérieur à celui obtenu pour l'extérieur de la coquille. Le nombre de bandes opaques annuelles, qu'on distingue des bandes parasites par leur position dans le cycle saisonnier de $\delta^{18}O$ dans la coquille, concordait avec le nombre de bandes comptées dans les sections intérieures polies. Les bandes opaques causées par des événements annuels étaient soit non continues de l'extrémité en croissance à l'umbo, soit non continues jusqu'à leur fusion avec d'autres bandes près de la ligne palléale. C'est le compte des bandes opaques internes, à l'aide des méthodes décrites dans cette étude, qui a permis d'obtenir la meilleure estimation de l'âge réel d'*Elliptio complanata*.
[Traduit par la Rédaction]

Introduction

Since the early 1900s, opaque bands on the shell of the Unionidae family of molluscs have been interpreted as marking annual events (Isley 1914; Grier 1922). The acceptance of this interpretation has given researchers a simple and precise method for aging molluscs and estimating their growth rates. It has also opened up the possibility of exploiting these animals as biomonitors.

Validation of the annual occurrence of these opaque bands has been limited to species-specific capture and release studies (Negus 1966; Ghent et al. 1978; Fritz and Haven 1983; Neves and Moyer 1988) or to the examination of shell microstructure (Fritz and Lutz 1986; Fritz et al. 1990). Others have relied on the visual appearance of the opaque bands when separating annually produced bands from bands created by some nonannual event (Tevesz and Carter 1980; McCuaig and Green 1983; Day 1984; Hinch and Stephenson 1987; Dettman and Lohmann 1993). Studies that determined the age of *Elliptio complanata* (Strayer et al. 1981; Hinch and Stephenson 1987; Metcalf-Smith and Green 1992) considered that the thickest, most opaque bands were annually produced, while thinner, less opaque bands were deemed to have been produced by some nonannual event. Although the latter technique involves some subjectivity, researchers have used and continue to use opaque bands to age and to study growth in a variety of unionid species (Magnin and Stanczykowska 1971; Day 1984; McCuaig and Green 1983; Hanson et al. 1988; Bauer 1992; Metcalf-Smith and Green 1992).

A validation technique that is independent of species and growth patterns is the use of the seasonal cycle of $\delta^{18}O$ in the shells of mollusks. If opaque bands on the shell are true annual bands, then the variation in the isotopic composition of the shell material should follow predicted seasonal patterns. Epstein et al. (1953) and Mook and Vogel (1968) showed that the oxygen in biogenic calcite of marine mollusc shells was deposited in isotopic equilibrium with the oxygen of the surrounding medium, and Stuiver (1970) and Fritz and Poplawski (1974) reached the same conclusion for freshwater species.

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However, because of isotopic fractionation, the ^{18}O equilibrium value of the $CaCO_3$ portion of the shell is directly dependent on the temperature and the ^{18}O content of the precipitating medium (e.g., the lake water) at the time of $CaCO_3$ precipitation (Epstein et al. 1953). Warm water temperatures result in shell that is depleted in ^{18}O compared with shell deposited when water temperatures are cold. This simple relationship between water temperature and the $\delta^{18}O$ of the shell becomes more complicated if evaporation causes the lake water to become enriched in ^{18}O (Dettman and Lohmann 1993). However, if there is no change in the isotopic composition of the water with time, or if the temperature effect on the shell material greatly exceeds any evaporation effects (for which a correction can be applied), then the shell material will be an ideal biological thermometer.

Sequential sampling across the lines of growth in a mussel shell reveals a sine wave pattern of $\delta^{18}O$ concentrations. Wave peaks correspond to thermal minima and troughs correspond to thermal maxima (Krantz et al. 1984, 1987; Wefer and Berger 1991; Dettman and Lohmann 1993). This suggests that a detailed examination of the $\delta^{18}O$ pattern in a shell will allow an investigator to pinpoint the time of deposition of the opaque bands and separate bands produced annually from those produced by nonannual events.

To make an objective interpretation of the banding both on the external surface and in internal sections of *E. complanata* shells, three hypotheses were tested. (*i*) The number of opaque bands on the shell's exterior equals the number of opaque bands counted in polished sections of shell. (*ii*) Annual bands were formed near the animal's thermal minimum (either at the onset of winter or at the start of growth in the spring), causing the $CaCO_3$ in an annual line to be enriched in ^{18}O. (*iii*) Annual bands identified by their $\delta^{18}O$ values would be the thickest, most opaque bands only.

Methodology

Specimens used in this study were collected from a small shallow cove on the eastern shore of Head Lake (Fig. 1), located in south-central Ontario on the southern boundary of the Canadian Shield, 44°43′N, 78°56′W. The lake is slightly alkaline (pH 7.9 to 8.3; Campbell and Evans 1991; Veinott 1994) and classified as mesotrophic (Ontario Ministry of the Environment 1972).

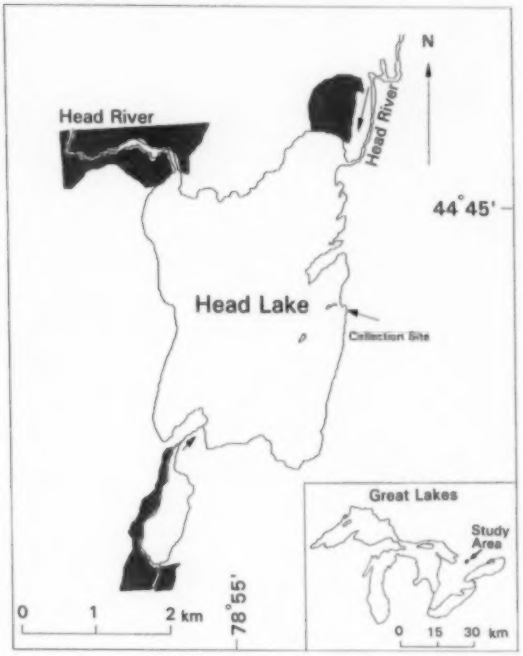
Two hundred and ninety live mussels ranging in length from 26 to 78 mm (axis of maximum growth) were collected by hand with the aid of a glass-bottom bucket. Collections were made in October 1991, June to October 1992, and April and May 1993. Approximately 40 mussels were collected each month with the exception of September 1992 when only 22 specimens were collected. Water depth rarely exceeded 1 m. All specimens were either partially exposed above the substrate or buried at the end of a trail visible in the soft bottom.

Specimens were transported to the laboratory, immediately rinsed in tap water, and killed by freezing at −14°C. Specimens remained frozen until shucked, then they were scrubbed clean with a plastic scouring pad, air dried, and stored at room temperature until analyzed.

Shell markings were described according to the convention described by Casselman (1983) who used the terms opaque and translucent when describing light and dark bands in calcified biological structures. Opaque bands are impervious to light and appear dark when viewed with transmitted light. Translucent zones transmit light (Casselman 1983).

To examine external markings, all shells were viewed with transmitted light (a 12-V 50-W tungsten lamp) without magnification.

Fig. 1. Map of Head Lake showing the collection site. Shaded areas indicate bogs.



Opaque bands on the shell exterior extending circumferentially from the umbo and occurring on both valves were considered to represent the end of one growing season. Every opaque band was assumed to be annual unless a shell was damaged or deformed, suggesting an alternative cause. The number of bands missing because of erosion of the umbo region was estimated on the basis of the average growth rate of juvenile mussels. The number of opaque bands counted by this method was referred to as the external age.

To examine internal markings, 79 shells ranging in length from 26 to 78 mm were cut longitudinally from the umbo to the growing edge along the axis of maximum growth on a Buehler Isomet low-speed saw with a diamond wafering blade. Sections of shell 500 µm thick were removed, lightly polished by hand with 1200 grit sand paper, and glued to a microscope slide with epoxy resin. The sections were reduced to approximately 200 µm thick by manually polishing with successively finer grit lapidary film (down to 10 µm) until a high gloss was achieved. Sections were viewed using a light microscope at 100× magnification and opaque bands were counted. Deciding what to count as an annual band became more difficult in polished sections because of the enhanced detail, varying line structure, and degrees of opacity. We standardized the analyses by starting at the tip of the growing edge and looking for opaque discontinuities in the prismatic layer (Fig. 2). If an opaque band extended from the prismatic layer discontinuity to the umbo region, or extended from the prismatic layer and merged into a line common to several opaque bands, it was counted as annual, regardless of its degree of opacity. Broken, noncontinuous bands were not counted. The number of opaque bands counted by this method was referred to as the internal age.

Shell material for ^{18}O analyses was sampled using the drilling technique and microsampling apparatus described by Dettman and Lohmann (1993). Briefly, photomicrographs of the polished, 200 µm thick sections of shell were used to select areas for microsampling. The areas selected were digitized. Points visible on both the photomicrographs and the polished shell sections were used as reference

Fig. 2. Photomicrograph of a section of shell showing prismatic layer (*pl*), prismatic layer discontinunity (*pld*), nacreous layer (*nc*), and opaque bands (*ob*). Growth is to the left.

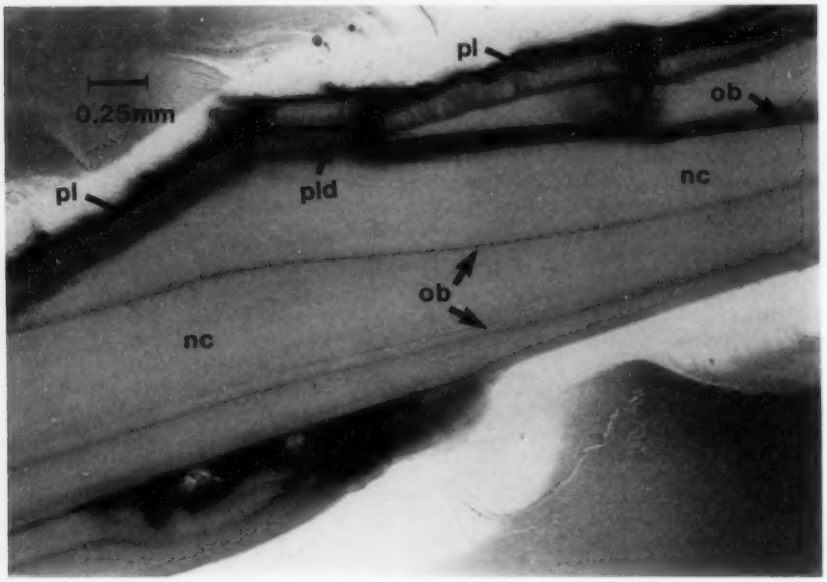


Table 1. Number of opaque bands (age) and length of *Elliptio complanata* shells used for $\delta^{18}O$ analyses.

Name	Internal age	External age	Length (mm)
Alpha	4	4	43
Beta	14	8	67
Tau	20	17	70
Rho	5	5	58
Omega	18	14	64

points to convert digitized curves on the photomicrographs to real sampling paths on the shell using a Cartesian transformation. The transformed data were used to control a low-speed drill. The drill bit was held stationary while the specimen was moved in the *x*, *y*, and *z* directions by stepping motors capable of 1-μm increments, and shell was milled from the specimen.

Five shells (alpha, beta, tau, rho, and omega) collected in July and August 1992 were selected for digitizing, sampling, and isotopic analyses. Ages and lengths are given in Table 1. Samples for isotopic analyses came from the inner nacreous layer only. To minimize mixing of material from different growth periods, sequential paths approximately 50 μm deep, 25 μm wide, and 3–5 mm long were milled parallel to the lines of growth. Milling began at the more recently produced shell material. The shell material (approximately 10–30 μg for each drill pass) was collected on the tip of a scalpel blade. If a single pass did not produce sufficient material for analyses, material from two or more sequential passes was combined. As shell was milled and removed, milling proceeded toward and across the opaque bands. Pass number and position in the shell were noted to map future $\delta^{18}O$ values.

A CarboKiel automated reaction device liberated CO_2 from each $CaCO_3$ sample. The CO_2 was then analyzed for $\delta^{18}O$ on a Finnigan MAT 251 mass spectrometer (Dettman and Lohmann 1993). Mean ratios were calculated and final values were referenced to the PDB (Peedee Belemnite) standard and given in δ notation (Rye and Sommer 1980): $\delta = ((R_{sa} - R_{st})/R_{st}) \times 1000$, where R_{sa} and R_{st} are the measured $^{18}O/^{16}O$ ratios of the sample and the reference standard, respectively. The precision of the $\delta^{18}O$ analysis is ±0.1‰ (Dettman and Lohmann 1993).

To define the oxygen isotopic concentration of water in Head Lake, water was collected approximately biweekly from March to September 1993. Water was pumped from approximately 5 to 10 cm above the sediment into clean 60-mL borosilicate glass bottles that had been rinsed in lake water and filled to overflowing. Several milligrams of crystalline mercuric chloride were immediately added to poison the sample and the bottle was sealed with a threaded cap with a conical polyseal insert. Water samples were stored at room temperature until analyzed for $\delta^{18}O$ according to the procedure of Drimmie and Heemskerk (1993).

Predicted shell $\delta^{18}O$ was calculated by the following equations of Dettman and Lohmann (1993): $10^3 \ln\alpha_{arag-water} = -0.2160T + 34.959 = \delta^{18}O_{arag}$ (SMOW) − $\delta^{18}O_{water}$ (SMOW); and $\delta^{18}O$ (SMOW) = 1.03091 × $\delta^{18}O$ (PDB) + 30.91. *T* is the temperature in degrees Celcius. SMOW (standard mean ocean water) and PDB are the two reference standards against which the isotopic ratios were compared. The subscripts arag and water refer to $\delta^{18}O$ measurements made on shell and water, respectively. Arag is an abbreviation for aragonite, a $CaCO_3$ polymorph found in *E. complanata* shells. The aragonite–water fractionation factor ($\alpha_{arag-water}$) is equal to (δ_{arag} + 1000)/(δ_{water} + 1000).

Results

Band counting

The internal age was compared with the external age of 79 *E. complanata* shells collected from Head Lake (Fig. 3). A comparison of regression slopes and intercepts (Zar 1984) showed that there was no significant difference between the intercepts ($p > 0.2$). However, the slope of the external age line was greater than the slope of the internal age line ($p < 0.05$).

Fig. 3. Linear regressions through number of opaque bands counted on the shell exterior (open squares) and opaque bands counted in shell sections (solid triangles). Straight line equations and R^2 values are shown.

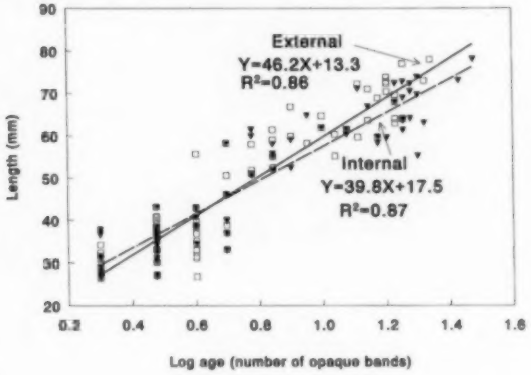
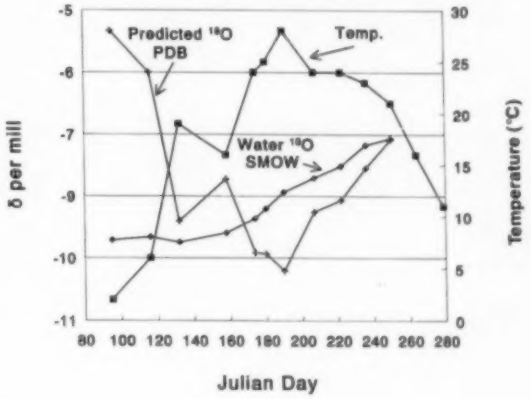


Fig. 4. Relationship between water temperature, water $\delta^{18}O$, and predicted shell $\delta^{18}O$ for Head Lake in 1993. SMOW, standard mean ocean water; PDB, Peedee Belemnite.



$\delta^{18}O$ values

The equations of Dettman and Lohmann (1993) predict Head Lake shell $\delta^{18}O$ values ranging from approximately –5.5 to –10‰ on the basis of 1993 water temperatures (Fig. 4). In general, however, our values for the $\delta^{18}O$ of the shell material did not reach these extremes. One possible explanation is that the shell material analyzed was produced during several different years and therefore that 1993 water temperatures are not good predictors. However, 1993 was not an anomalous year with respect to water temperatures (Veinott 1994). Other possibilities include a shorter than expected shell-producing season, a stoppage of growth during the hottest part of the year, or a microsampling resolution that was too coarse. Regardless of which of these hypotheses is correct, our model will still be valid and therefore the position of the opaque band in the $\delta^{18}O$ cycle can be used to identify annually produced bands.

Alpha was an easy shell to age. It had three distinct opaque bands on the shell exterior, all of which were complete. The most recently formed external band appeared as a sharp clear change in the shell's opacity when viewed with transmitted light in a polished longitudinal section (Fig. 5*a*). It was even possible to see the individual growth laminae as the line was approached from either side. Alpha band A met the criteria used in this study for including a band in the age of an animal, and it was interpreted as marking the beginning of growth of the 4th year.

Microsampling of alpha began at the most recently produced shell material (IS in Fig. 5*a*, and corresponding to distance 0 in Fig. 6*a*) and proceeded toward the prismatic layer (top of Fig. 5*a*). The isotopic profile (Fig. 6*a*) shows alpha band A forming not at the lightest (coldest temperature) $\delta^{18}O$ value measured (≅6.8‰) but with a $\delta^{18}O$ value of –7.7‰. This corresponds to a temperature of formation of approximately 13°C. The position of the band on the $\delta^{18}O$ profile suggests that this band was deposited in the spring.

Beta band A (Fig. 5*b*) also satisified the criteria to be labelled annual. However, the band was approximately twice as thick (≅100 μm) as alpha band A. The two bands were visually similar with respect to opacity. Beta band A included the lightest $\delta^{18}O$ value measured in this specimen (Fig. 6*b*); further, it had a lighter mean $\delta^{18}O$ value (≅1‰ lighter) than alpha band A. This suggests a colder temperature of formation for beta band A. However, the data did show decreasing $\delta^{18}O$ values as band A was forming, suggesting that the band was formed in the spring as the water temperature increased, in agreement with the data from alpha.

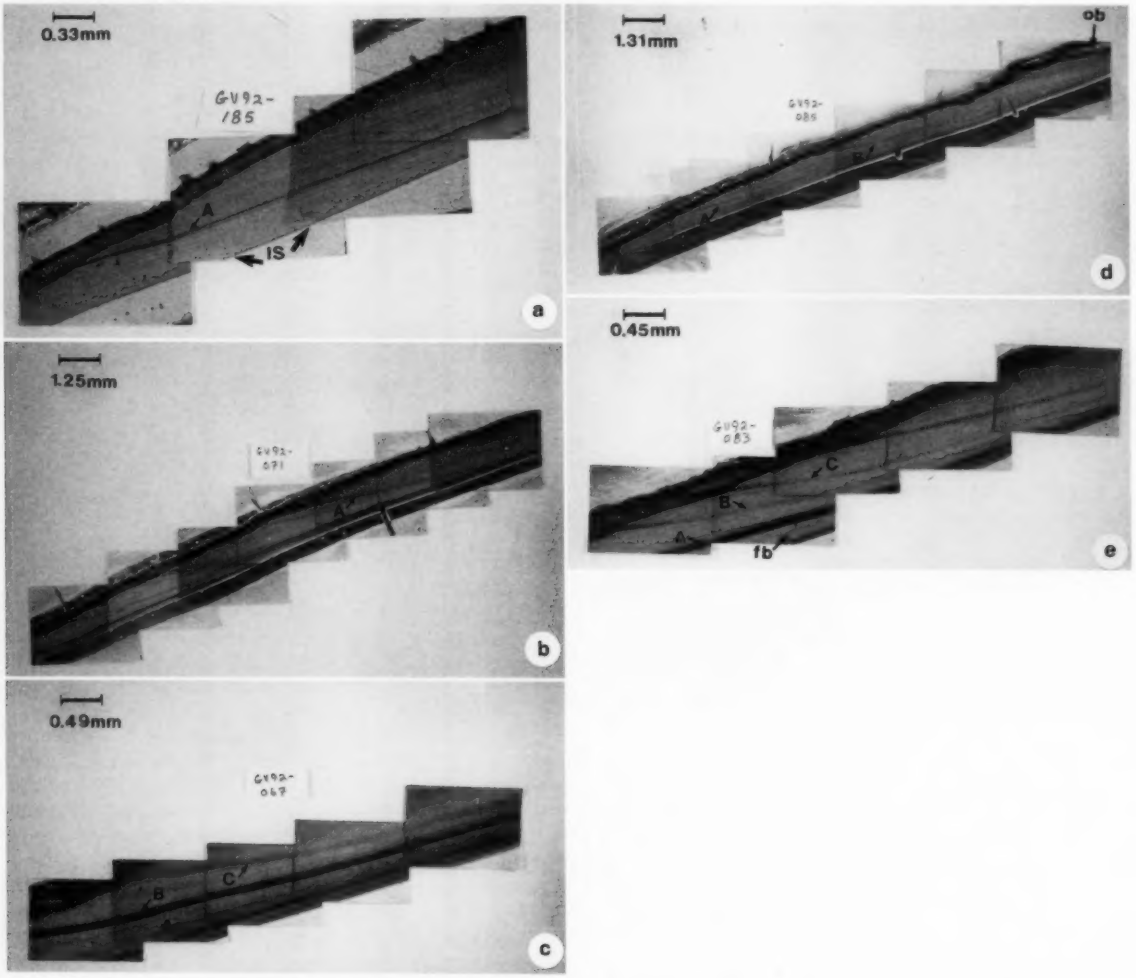
Tau was microsampled across three annual bands (Fig. 5*c*). Bands B and C both formed parts of very distinct $\delta^{18}O$ peaks (Fig. 6*c*) with similar amplitudes with less than 0.5‰ difference in their average $\delta^{18}O$ concentration. However, the degree of opacity of these two bands was quite different. Bands A and B were visually more similar than bands B and C, but band A was not part of an isotopic peak. This suggests that there is not always a direct correlation between the appearance of opaque bands and the season during which they are deposited.

The fourth specimen (rho) had what appeared, a priori, to be two successive annually produced bands (Fig. 5*d*) that were lighter (less opaque) and narrower than most of the other internal bands in this specimen (cf. opaque band (*ob*) at far upper right corner of Fig. 5*d*). They were also less opaque than most of the bands in alpha, beta, or tau.

Bands A and B of rho were at or very near a $\delta^{18}O$ maximum concentration, although there was a gap in the profile in Fig. 6*d*. Regardless of the value of the missing determination, however, the results suggest that both bands were formed during a thermal minimum and both should be counted when aging the animal.

The specimen omega (Fig. 5*e*) had an opaque band (band A) that did not meet the criteria used here to identify an annual band. The band was not continuous from the prismatic layer to a common opaque band near the pallial line. The band was broken near the prismatic layer (not shown in Fig. 5*e*). It was, however, quite thick and very opaque. The position of omega band A in the $\delta^{18}O$ cycle suggests that this band was formed in late summer (Fig. 6*e*) and is the only band analyzed to have a constant $\delta^{18}O$ concentration through the entire opaque band. Although no band appeared on the $\delta^{18}O$ peak near band A (Fig. 6*e*) there was a very faint band (*fb*, visible in Fig. 5*e*) near this peak. It was not marked in Fig. 6*e* because it was not specifically isolated for analysis during digitizing. Two other

Fig. 5. (*a*) Photomicrograph composite of shell alpha. The letter A identifies the opaque band that was microsampled. IS is the inner surface of the shell. Growth is to the left. (*b*) Photomicrograph composite of shell beta. The letter A identifies the opaque band that was microsampled. Growth is to the left. (*c*) Photomicrograph composite of shell tau. The letters A, B, and C identify the opaque bands that were microsampled. Growth is to the left. (*d*) Photomicrograph composite of shell rho. The letters A and B identify the opaque bands that were microsampled. *ob* identifies an opaque band that was not microsampled. Growth is to the left. (*e*) Photomicrograph composite of shell omega. The letters A, B, and C identify the opaque bands that were microsampled. *fb* indicates a faint band that was not sampled. Growth is to the left.

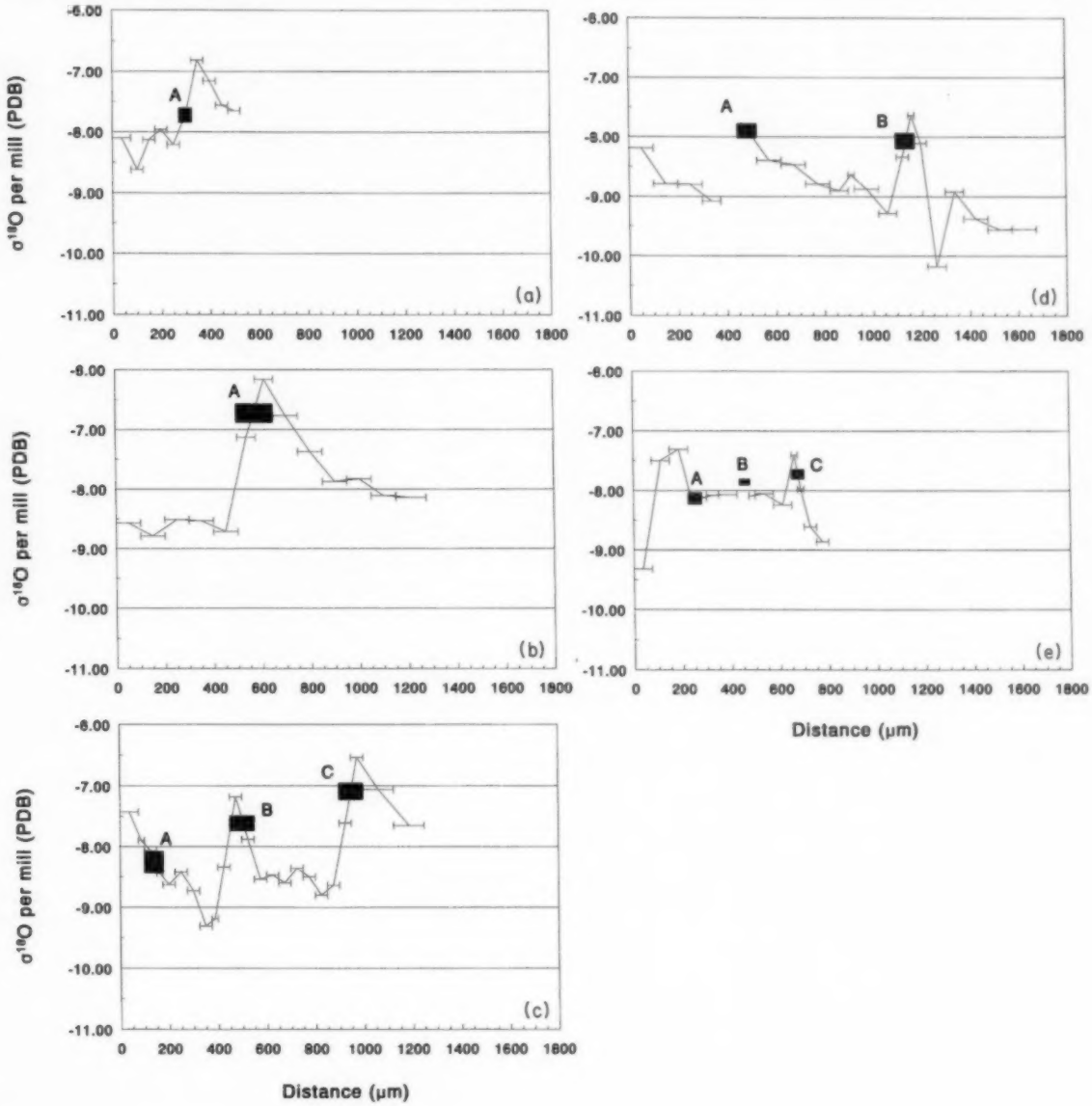


bands (B and C), both of which met the criteria for annual bands, were also sampled. The material removed from band B (Fig. 6*e*) was lost because of equipment failure. Band C was approximately 50 μm wide with an average $\delta^{18}O$ concentration (–7.7‰) near the $\delta^{18}O$ maximum. The result from band C is consistent with measurements made on other bands from the other shells. Therefore, bands B and C should be considered annual bands when aging this animal, and band A, because it was incomplete, should be considered a disturbance band.

Discussion

External ages tended to be less than internal ages. These results agree with those of Neves and Moyer (1988) who concluded that, on average, more bands were counted in shell cross sections than on the shell exterior. Therefore, either annual bands were not being counted on the exterior of the shell, or disturbance bands were being included in the internal age estimates. Our data support the former idea. Every opaque band (with the exception of tau band A) analyzed for $\delta^{18}O$ that met our criteria for inclusion in the internal age of the shell was produced at or near the thermal minimum. The results from omega band B were inconclusive because data were missing. This regular production of opaque bands and the lack of opaque bands in different positions of the $\delta^{18}O$ cycle strongly support the idea that these bands are produced annually and are not a result of some disturbance event.

Fig. 6. $\delta^{18}O$ profiles of shells alpha (*a*), beta (*b*), tau (*c*), rho (*d*), and omega (*e*). In each panel, the dark boxes and uppercase letters correspond to the opaque band in the photomicrograph of the shell with the same name. The dark boxes also show the position and width of the opaque band and are centered vertically on the mean $\delta^{18}O$ concentration in the band. Horizontal bars represent the width of the path milled from the shell.



The change in $\delta^{18}O$ values as the opaque band forms suggests that the opaque bands are growth structures. The opacity may be a result of a change in the microstructure of the shell (Day 1984; Fritz and Lutz 1986; Fritz et al. 1990). However, the hypothesis that the width and degree of opacity of the bands are appropriate criteria for separating annually produced bands from bands produced by nonannual events was not supported by our data. Alpha, beta, and rho produced bands of differing thickness (40–100 μm) yet all formed near the thermal minimum. The difference in band formation temperature between alpha and beta and the difference in the amplitude between the two $\delta^{18}O$ profiles (0.82‰) may be a reflection of the different ages of the shells. Alpha and beta were judged to be 3 and 14 years old, respectively (internal age), and were 43 and 67 mm long, respectively. Beta may have begun shell deposition earlier (and at a colder temperature) in the spring than alpha. However, this does not agree with Harrington (1989) or Fritz and Haven (1983) who suggested that inactive

periods increased as individuals matured, resulting in a later start of metabolic activity in the spring.

Tau produced opaque bands with strikingly different visual appearances (compare A and B in Fig. 5*c* with C). However, the visually similar bands (A and B) were not isotopically similar (see Fig. 6*c*). Therefore, the visual appearance of the bands is not a good predictor of isotopic values. However, bands A, B, and C met our criteria for inclusion in the age of the animal, but the isotopic data suggest that band A is not an annual band. Therefore, we have incorrectly classified band A as an annual band. However, this was the only incorrect classification. Attempts to group bands on the basis of their degree of opacity or thickness necessitate the exclusion of obvious isotopic peaks. That will result in (assuming that the $\delta^{18}O$ cycle is the most accurate estimate of the age of these animals) less accurate age estimates than the age estimates given in this paper.

On average the number of opaque bands counted on the exterior of *E. complanata* shells was less than the number of opaque bands counted in polished longitudinal sections of shell. Using the opaque bands on the exterior of *E. complanata* shells to determine the age or growth rate will underestimate age and overestimate growth rate. The errors in age estimates based on the number of external opaque bands will increase as animals mature.

Seven of eight continuous internal opaque bands that were sampled for $\delta^{18}O$ were formed at the lowest temperature at which the mussels deposited shell. Comparisons of the $\delta^{18}O$ of internal bands of varying thickness (40–100 μm) and opacity (very light to black) showed that all continuous internal opaque bands seen in polished, longitudinal sections should be regarded as annual lines. Only broken or discontinuous opaque bands should be regarded as marking some nonannual event. On the basis of these conclusions we reject hypotheses 1 and 3 and accept hypothesis 2.

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References

Bauer, G. 1992. Variation in the life span and size of the freshwater pearl mussel. J. Anim. Ecol. **61**: 425–436.

Campbell, J., and Evans, R.D. 1991. Cadmium concentrations in the fresh water mussel (*Elliptio complanata*) and their relationship to water chemistry. Arch. Environ. Contam. Toxicol. **20**: 125–131.

Casselman, J.M. 1983. Age and growth of fish from their calcified structures: techniques and tools. NOAA Tech. Rep. NMFS No. 8.

Day, M.E. 1984. The shell as a recording device: growth record and ultra structure of *Lampsilis radiata* (Pelecypoda: Unionidae). Can. J. Zool. **62**: 2495–2504.

Dettman, D.L., and Lohmann, K.C. 1993. Seasonal change in paleogene surface water $\delta^{18}O$: fresh-water bivalves of western North America. *In* Climate change in continental isotopic records. AGU Geophysical Monograph Vol. 78. *Edited by* P.K. Swart, K.C. Lohmann, J. McKenzie, and S. Savin. American Geophysical Union, Washington, D.C. pp. 153–163.

Drimmie, R.J., and Heemskerk, A.R. 1993. Water ^{18}O by CO_2 equilibration. Technical procedure 13.0, revision 02. Department of Earth Sciences, University of Waterloo, Waterloo, Ont.

Epstein, S.R., Buchsbaum, R., Lowenstam, H.A., and Urey, H.C. 1953. Revised carbonate-water isotopic temperature scale. Geol. Soc. Am. Bull. **64**: 1315–1325.

Fritz, L.W., and Haven, D.S. 1983. Hard clam, *Mercenaria mercenaria*: shell growth patterns in Chesapeake Bay. Fish. Bull. **81**: 697–708.

Fritz, L.W., Ragone, L.M., and Lutz, R.A. 1990. Microstructure of the outer shell layer of *Rangia cuneata* (Sowerby, 1831) from the Delaware River: applications in studies of population dynamics. J. Shellfish Res. **9**: 205–213.

Fritz, L.W., and Lutz, R.A. 1986. Environmental perturbations reflected in internal shell growth patterns of *Corbicula fluminea* (Mollusca: Bivalvia). Veliger, **28**: 401–417.

Fritz, P., and Poplawski, S. 1974. ^{18}O and ^{13}C in the shells of freshwater mollusks and their environment. Earth Planet. Sci. Lett. **24**: 91–98.

Ghent, A.W., Singer, R., and Johnson-Singer, L. 1978. Depth distributions determined with SCUBA, and associated studies of the freshwater unionid clams *Elliptio complanta* and *Anodonta grandis* in Lake Bernard, Ontario. Can. J. Zool. **56**: 1654–1663.

Grier, N.M. 1922. Observations on the rate of growth of the shell of the lake-dwelling fresh-water mussels. Am. Midl. Nat. **8**: 129–148.

Hanson, J.M., Mackay, W.C., and Prepas, E.E. 1988. Population size, growth, and production of a unionid clam, *Anodonta grandis simpsoniana*, in a small, deep Boreal Forest lake in central Alberta. Can. J. Zool. **66**: 247–253.

Harrington, R.J. 1989. Aspects of growth deceleration in bivalves: clues to understanding the seasonal $\delta^{18}O$ and $\delta^{13}C$ record — a comment on Krantz et al. (1987). Palaeogeogr. Palaeoclim. Palaeoecol. **70**: 399–407.

Hinch, S.G., and Stephenson, L.A. 1987. Size and age-specific patterns of trace metal concentrations in freshwater clams from an acid sensitive circumneutral lake. Can. J. Zool. **65**: 2436–2442.

Isley, F.B. 1914. Experimental study of the growth and migration of fresh-water mussels. Appendix III. Rep. U.S. Comm. Fish.

Krantz, D.E., Jones, D.S., and Williams, D.F. 1984. Growth rates of the sea scallop, *Placopecten magellanicus*, determined from the $^{18}O/^{16}O$ record in shell calcite. Biol. Bull. (Woods Hole), **167**: 186–199.

Krantz, D.E., Williams, D.F., and Jones, D.S. 1987. Ecological and paleoenvironmental information using stable isotope profiles from living and fossil molluscs. Palaeogeogr. Palaeoclim. Palaeoecol. **58**: 249–266.

Magnin, E., and Stanczykowska, A. 1971. Quelques données sur la croissance, la biomasse et la production annuelle de trois mollusques Unionidae de la région de Montréal. Can. J. Zool. **49**: 491–497.

McCuaig, J.M., and Green, R.H. 1983. Unionid growth curves derived from annual rings: a baseline model for Long Point Bay, Lake Erie. Can. J. Fish. Aquat. Sci. **40**: 436–442.

Metcalf-Smith, J.L., and Green, R.H. 1992. Ageing studies on three species of freshwater mussels from a metal-polluted watershed in Nova Scotia, Canada. Can. J. Zool. **70**: 1284–1291.

Ontario Ministry of the Environment. 1972. Report on the water quality of Head Lake. Ontario Ministry of the Environment, Toronto, Ont.

Mook, W.G., and Vogel, J.C. 1968. Isotopic equilibrium between shells and their environment. Science (Washington, D.C.), **159**: 874–875.

Negus, C.L. 1966. A quantitative study of growth and production of unionid mussels in the River Thames at Reading. J. Anim. Ecol. **35**: 513–532.

Neves, R.J., and Moyer, S.N. 1988. Evaluation of techniques for age

determination of freshwater mussels (Unionidae). Am. Malacol. Bull. **6**: 179–188.

Rye, D.M., and Sommer, M.A., II. 1980. Reconstructing paleotemperature and paleosalinity regimes with oxygen isotopes. *In* Skeletal growth of aquatic organisms. *Edited by* D.C. Rhoads and R.A. Lutz. Plenum Press, New York. pp. 169–202.

Strayer, D.L., Cole, J.J., Likens, G.E., and Buso, D.C. 1981. Biomass and annual production of the freshwater mussel *Elliptio complanata* in an oligotrophic soft water lake. Freshwater Biol. **11**: 435–440.

Stuiver, M. 1970. Oxygen and carbon isotope ratios of fresh-water carbonates as climatic indicators. J. Geophys. Res. **75**: 5247–5257.

Tevesz, M.J.S., and Carter, J.G. 1980. Environmental relationships of shell form and structure of unionacean bivalves. *In* Skeletal growth of aquatic organisms. *Edited by* D.C. Rhoads and R.A. Lutz. Plenum Press, New York. pp. 295–322.

Veinott, G.I. 1994. Aging and identification of annually produced opaque bands in the shell of the freshwater mussel *Elliptio complanata* using ^{14}C, $\delta^{13}C$ and $\delta^{18}O$. M.Sc. thesis, Trent University, Peterborough, Ont.

Wefer, G., and Berger, W.H. 1991. Isotope paleontology: growth and composition of extant calcareous species. Mar. Geol. **100**: 207–248.

Zar, J.H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, N.J.

Stock identification of chinook salmon (*Oncorhynchus tshawytscha*) using minisatellite DNA variation

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Abstract: Geographic variation in minisatellite DNA was examined in 28 stocks of chinook salmon (*Oncorhynchus tshawytscha*) from British Columbia and 3 stocks from the Yukon Territory. Genomic DNA was restricted with the enzymes *Rsa*I and *Hin*fI and hybridized with three minisatellite probes. Regional differentiation in allele frequencies and DNA fragment (band) counts was observed, with stocks from the Fraser River, east coast of Vancouver Island, west coast of Vancouver Island, southern mainland, and northern British Columbia forming distinct groups. With allele frequencies or band counts in the baseline stocks considered fixed or exact, estimates of stock composition from simulated mixtures within the Fraser and Skeena River drainages were accurate and precise, suggesting that discrimination among stocks within river drainages may be possible. Individual chinook salmon were classified with an average accuracy of 35% to stock in a 30-stock baseline and an average accuracy of 65% to region for eight regions. Minisatellite DNA variation offers the potential to accurately and precisely estimate chinook salmon stock composition on a fine geographic scale.

Résumé : On a examiné les variations géographiques d'ADN minisatellite dans 28 stocks de saumon quinnat (*Oncorhynchus tshawytscha*) de la Colombie-Britannique et 3 stocks du territoire du Yukon. L'ADN génomique était restreint par les enzymes *Rsa*I et *Hin*fI et hybride avec trois segments d'ADN minisatellite employés comme sondes. On a observé une différenciation selon la région des fréquences des allèles et des comptes de fragments (bandes) d'ADN chez les stocks du fleuve Fraser, de la côte est de l'île de Vancouver, de la côte ouest de cette même île, du sud de la partie continentale et du nord de la Colombie-Britannique, qui formaient des groupes distincts. Si l'on supposait que les fréquences des allèles ou les comptes des bandes des stocks de base étaient fixes ou exactes, les estimations de la composition des stocks faites à partir de mélanges simulés dans les bassins hydrographiques du Fraser et de la rivière Skeena étaient exactes et précises, ce qui suggère que la discrimination entre les stocks d'un même bassin hydrographique pourrait être possible. Les spécimens de saumon quinnat étaient classés avec une exactitude moyenne de 35% pour le stock (30 stocks de base) et avec une exactitude moyenne de 65% pour la région (huit régions). La variation de l'ADN minisatellite rend possible une évaluation exacte et précise de la composition des stocks de saumon quinnat sur une échelle géographique fine.
Traduit par la Rédaction]

Introduction

Chinook salmon (*Oncorhynchus tshawytscha*) support major recreational and commercial fisheries in the Pacific northwest, and effective management of these fisheries requires that stock composition of catches be determined to avoid excessive exploitation of selected stocks. Considerable effort has thus been directed towards stock identification. Coded-wire tags (Jefferts et al. 1963) are the principal means of estimating stock composition of chinook salmon catches. Coded-wire tags are relatively easy to apply, and tagging of juveniles reared in hatcheries is a routine practice. However, tagging of wild juveniles has usually been unsuccessful, and the reliability of the estimates of stock composition derived from coded-wire tags is sensitive to marking and sampling intensity (Shaul and Clark 1990; Geiger 1990), as well as to assumptions about the distribution in the fishery of unmarked stocks.

Stock identification techniques based upon genetic variation have been increasingly applied in salmon fisheries. Variation at protein-coding loci has been widely used to determine population structure (Winans 1989; Utter et al. 1989; Beacham et al. 1989) and estimate stock compositions in salmon fisheries (Beacham et al. 1985; Utter et al. 1987; Wood et al. 1989; Shaklee et al. 1990). Differences in allele frequencies among stocks for a number of loci are used to estimate stock compositions in fishery samples, generally using a maximum likelihood technique (Milner et al. 1981; Fournier et al. 1984). Individual fish are not identified to specific stocks, but rather allele or genotypic frequencies at a number of loci in the sample are used to estimate the most probable stock composition.

The ability to screen genetic variation at the DNA level has been used to investigate population structure in fish (Kornfield and Bogdanowicz 1987; Wilson et al. 1987; Grewe and Hebert 1988; Ward et al. 1989; Palva et al. 1989; Bermingham et al. 1991; Bernatchez et al. 1992; Taylor 1995), but applications to estimation of stock composition in Pacific salmon fisheries have been limited. Variation in the nuclear DNA of chinook salmon detected by a single probe has been used to distinguish

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farmed (largely derived from Big Qualicum River stock) from wild salmon on the west coast of Vancouver Island in British Columbia (Withler et al. 1994). Variation in minisatellite DNA has been used to determine the region of origin of individual chum salmon (*Oncorhynchus keta*) (Taylor et al. 1994) or the population of origin for some chinook salmon populations (Heath et al. 1995), as well as provide accurate and precise estimates of stock composition in simulated mixtures for some *Oncorhynchus* species (Beacham et al. 1995; Miller et al. 1996).

Minisatellite DNA consists of arrays of tandemly repeated nucleotide sequences with each array containing a distinctive core sequence of between 10 and 75 nucleotides (Jarman and Wells 1989). Variable numbers of the repeated core sequence occur between restriction sites, resulting in allelic variation. These minisatellite loci can be quite variable in salmonids, with heterozygosities reported between 35 and 80% (Taggart and Ferguson 1990; Bentzen and Wright 1993; Taylor et al. 1996). This level of variation may enhance stock discrimination, and thus increase the accuracy and precision of estimates of stock composition.

Estimation of the stock composition of a fishery sample is the primary objective of most stock identification efforts, but a more difficult task is that of identifying the individual fish in a sample to specific stocks. In the current study, we surveyed DNA variation in chinook salmon with three minisatellite DNA probes and evaluated the utility of the observed variation for estimation of chinook salmon stock composition and for classification of individuals to specific stocks or regions. Stock composition for simulated fishery samples from major river systems (Fraser and Skeena rivers in British Columbia) was estimated to evaluate the utility of applying minisatellite DNA variation for management of local stock identification issues. Potential applications within a river drainage were investigated because stocks within a drainage tend to be more similar genetically to each other than to stocks in more distant areas; thus, examination of the ability to discriminate among closely related stocks provides a good test of the utility of minisatellite DNA variation in practical fishery management problems.

Materials and methods

DNA samples

Liver samples were collected from spawning adult chinook salmon from 28 stocks in British Columbia (Fig. 1) and 3 stocks in the Yukon Territory during 1987–1993. Locations for the Yukon Territory stocks (Yukon mainstem (29), Whitehorse hatchery (30), Teslin River (31)) were outlined by Beacham et al. (1989). The term stock is used in the sense of Ricker (1972), in which a stock represents a discrete breeding unit spawning at a given time or place in a river or lake. The methods of DNA extraction have been outlined by Withler et al. (1994) and Beacham et al. (1995).

Probes

In this study, we used the probe Ssa1, a minisatellite repeat probe (core sequence 16 base pairs (bp)) isolated from Atlantic salmon (*Salmo salar*) (Bentzen et al. 1993). The probe was nonradioactively labelled using the random primer method of Feinberg and Vogelstein (1983) by incorporation of the nucleotide analogue digoxigenin-11-dUTP (Bronstein et al. 1990). We also used the probe pSsa-A34, a hypervariable minisatellite single locus probe isolated from Atlantic salmon (Taggart and Ferguson 1990). The final probe that we used was OtsPBS-1, isolated in our laboratory from a chinook salmon phagemid library. The library was constructed by extracting chinook salmon genomic DNA, restricting it with *Bam*HI, and ligating all fragments less than 2.0 kilo base pairs (kbp) into the phagemid vector pBluescript II (Stratagene). Recombinant phagemids were recovered by transformation into *Escherichia coli* DH5α-F′ cells (Gibro BRL) and plating under selective conditions for ampicillin resistance (Sambrook et al. 1989). Colonies were plate transferred to nylon membranes and probed with the radioactively labelled probe B2-2 originally derived from chinook salmon (Stevens et al. 1993). The DNA of putative positives was isolated using the Promega Magic Mini-prep kit (Promega Corporation, Madison, Wis.). The clones were radioactively labelled and used to probe membranes containing *Bam*HI-restricted chinook salmon genomic DNA. A variable banding pattern was produced with clone OtsPBS-1. Both strands of the clone were subsequently sequenced (Table 1). This probe is 196 bp in length and is composed of five imperfect repeats of a 40-bp sequence. The probe was labelled with ^{32}P by the method of Feinberg and Vogelstein (1983).

Southern blot analysis

Approximately 4.0 μg of genomic DNA per fish was digested with either the restriction enzyme *Hin*fI or *Rsa*I and loaded onto a 0.7% horizontal agarose gel. Each gel contained 30 samples, comprised of four lanes of *Hin*dIII-restricted lambda DNA size markers, one lane of the standard fish sample that was run on each gel (BQ12), and 25 lanes of samples from the populations surveyed. Three populations were loaded on each gel, with eight or nine individuals examined for each population. The DNA was electrophoresed at 40 V for 20 h. The DNA was transferred onto Hybond N nylon membranes (Amersham, Arlington Heights, Ill.) by vacuum blotting and bound to the membrane by UV cross-linking (Sambrook et al. 1989). The hybridization procedure was an adaptation of the technique of Westneat et al. (1988). The membranes for both restriction enzymes were incubated in 0.10 mL/cm^2 prehybridization solution (7.0% sodium dodecyl sulphate (SDS); 1.0 mM ethylenediaminetetraacetic acid (Na_2EDTA), pH 8.0; 1.0% bovine serum albumin (BSA), fraction V; 0.263 M Na_2HPO_4) at 65°C for at least 2 h. This solution was then replaced with 0.05 mL/cm^2 prehybridization solution and incubated for 16 h at 65°C. The stringency washes were as outlined by Beacham et al. (1995). DNA variation for Ssa1 was detected by chemiluminescence (Bronstein and McGrath 1989; Höltke et al. 1992) followed by exposure of the membrane to X-ray film at room temperature for between 2 and 48 h.

Following successful hybridization of the *Hin*fI and *Rsa*I membranes with Ssa1, the probe was stripped from the membrane by the high temperature method of Noppinger et al. (1992). The membrane containing *Rsa*I-digested DNA was hybridized with the pSsa-A34 probe and variation was detected by chemiluminescence as with Ssa1. The stringency washes were similar to those outlined by Beacham et al. (1995). The OtsPBS-1 probe was labelled with ^{32}P and hybridized to the *Hin*fI membranes. Autoradiography ranged from 4 h to several days at –85°C with intensifying screens.

Analysis of DNA blots

DNA autoradiographs were analyzed with Bioimage software (Millipore Corp. Imaging Systems, Ann Arbor, Mich.). Autoradiographs were scanned using a Kodak high-resolution two-dimensional charge coupled device (CCD) camera at a 1024 × 1024 pixel density. The resultant images were translated into digital image files and stored on a SPARC workstation for later analysis. Probing the membranes with the Ssa1 and OtsPBS-1 probes produced autoradiographs with bands of varying intensity. We scored only those bands that were dark and visibly discrete, ignoring bands that were too light to be reliably distinguished from background hybridization. The four lanes containing lambda DNA size markers (23.1, 9.4, 6.6, 4.4, 2.3, and 2.0 kbp) on each autoradiograph were used to create a molecular

Fig. 1. Locations in British Columbia where chinook salmon were sampled during 1987–1993. West coast of Vancouver Island: Conuma River (1), Robertson Creek (2), Kennedy River (3), Thornton Creek (4), Nitinat River (5). East coast of Vancouver Island: Cowichan River (6), Nanaimo River (7), Big Qualicum River (8), Quinsam River (9). Fraser River: Harrison River (10), Quesnel River (11), Nechako River (12), Stuart River (13), Salmon River (14), Dome Creek (15), Fraser River at Tete Jaune (16). Southern mainland: Squamish River (17), Bute Inlet (18), Wannock River (19). Northern mainland: Atnarko River (20), Kitimat River (21). Skeena River: lower Kitsumkalum River (22), upper Kitsumkalum River (23), Kitwanga River (24), Bear River (25), Bulkley River (26), Morice River (27). Nass River: Kwinageese River (28).

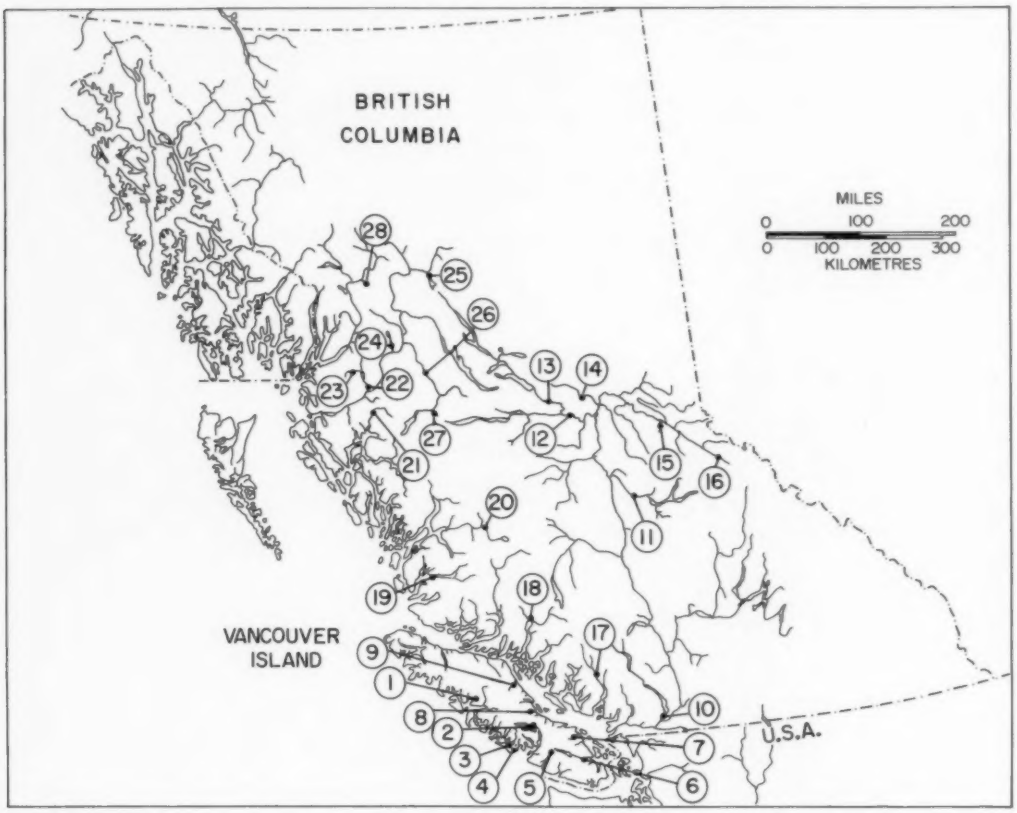


Table 1. The complete nucleotide sequence of probe OtsPBS-1 isolated from a chinook salmon.

Position	Sequence
1	A G G A C T A C A G T T G T T A G G T A G C T G T C T T T A A A T A G T G T C C
41	A G A G G A C T A T A G T T G T T G T A G C T G T C T T T A T A T A G G G T C C
81	A G G A C T A T A G T T G T T A G G T A G C T G T C T T T A T A T A T G G A G A
121	A G - A C T A T A G T T G T T A G G T A G C T G - - T T T C T A T A T A G T G T C
159	A G G A C T A T A G T T G T T A G G T A G - T G T C T T T A T A T A G G A T C

Note: Hyphens have been inserted to improve the alignment.

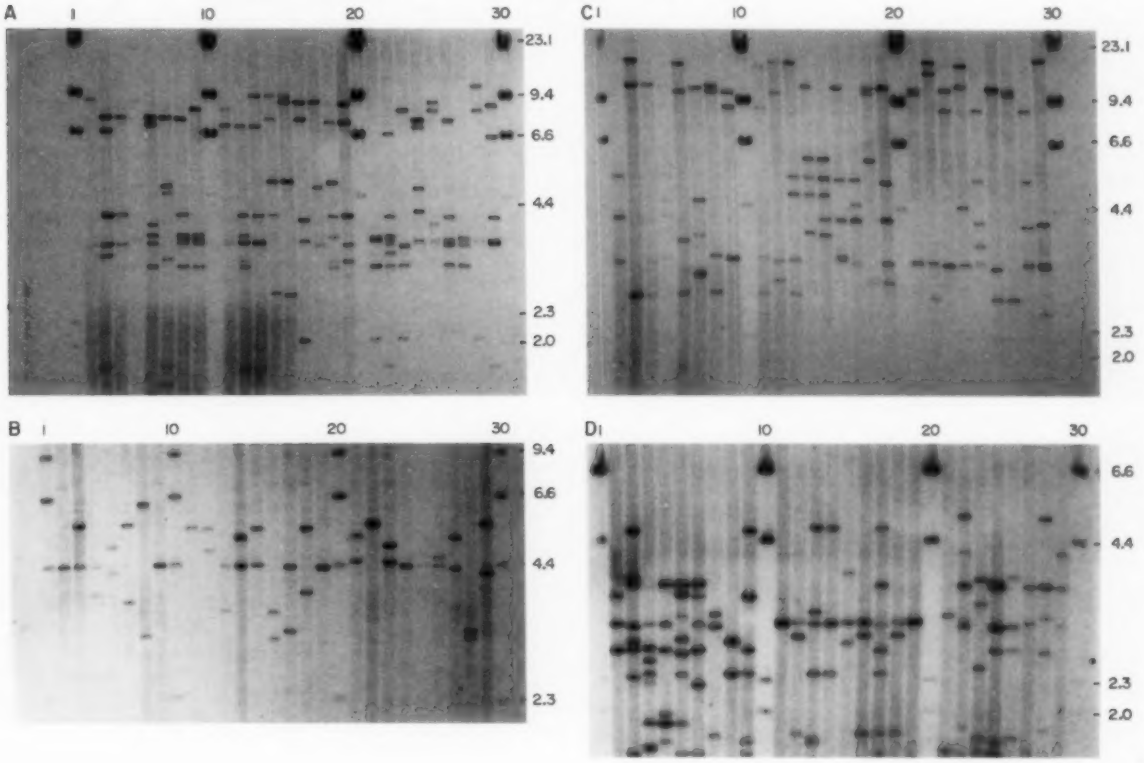
weight size standard network that spanned the whole blot. Molecular weights were then assigned by the software to each band in each lane. Bands between 2.0 and 23.1 kbp were scored on Ssa1 images, between 2.0 and 8.5 kbp on Ssa-A34 images, and between 2.0 and 7.0 kbp on OtsPBS-1 images.

Band interpretation

The pSsa-A34 probe detects both alleles of a single Mendelian locus in chinook salmon and provides hybridization patterns with one or two bands per fish (Stevens et al. 1993). Because we have not observed null alleles in pedigree analysis, we scored each band of double-banded patterns as an allele and the one band of single-banded patterns as two comigrating alleles. We determined allele frequencies for 19 Ssa-A34 alleles defined by binning (see below).

Analysis of pedigree data in chinook salmon (R.E. Withler and K. Miller, unpublished data) has indicated that the multibanded Ssa1 hybridization patterns reflect variation at a single, highly polymorphic locus as described for coho salmon (Miller et al. 1996). Thus, the

Fig. 2. Autoradiographs of chinook salmon restricted with *Rsa*I and hybridized with Ssa1 (A) and pSsa-A34 (B) and restricted with *Hinf*I and hybridized with Ssa1 (C) and OtsPBS-1 (D). Lanes are as follows: (A) 1, lambda standard; 2–9, Quinsam River; 10, lambda standard; 11–19, Atnarko River; 20, lambda standard; 21–28, Cowichan River; 29, standard fish BQ-12; 30, lambda standard; (B) 2–9, Big Qualicum River; 11–19, Nanaimo River; 21–28, Cowichan River; other lanes as in A; (C) all lanes as in A; (D) 2–9, lower Kitsumkalum River; 11–19, Wannock River; 21–28, Nanaimo River, other lanes as in A.



Ssa1 alleles contain internal restriction sites and produce cosegregating haplotypes on Southern blots. Because the locus is highly polymorphic, and many of the haplotypes produced from different alleles share restriction fragments of the same or similar sizes, it is not possible to score individual haplotypes and calculate Ssa1 allele frequencies, except for pedigreed individuals (Miller et al. 1996). Instead, we scored between 1 and 9, or 1 and 10, bands per fish for DNA restricted with *Rsa*I and *Hin*fI, respectively (Fig. 2). The mean observed band counts per fish were determined for each of 31 band size bins (see below), and no attempt was made to determine the level of heterozygosity at the Ssa1 locus.

OtsPBS-1 detects DNA fragments in chinook salmon that reflect variation at a minimum of two independent loci. Pedigree analysis has revealed the presence of null alleles, many of which may simply be fragments too small to be detected under the conditions of the present study (unpublished data). We scored between 0 and 7 bands in each fish between 2.0 and 7.0 kbp, and determined mean band counts for each of 22 band size bins (see below).

Band assignment

The DNA samples from fish in this study were run on multiple gels, making it necessary to determine the measurement error of band size among gels. The precision of our estimation of band size was determined by calculating the mean and standard deviation of the repeated size measurements of six Ssa1 *Hin*fI fragments, six Ssa1 *Rsa*I fragments, two pSsa-A34 *Rsa*I fragments, and three OtsPBS-1 *Hin*fI fragments of the standard fish BQ12 across gels. We regressed the standard deviation (SD) on fragment size (FS, in bp) ($n = 17$, $F = 193.1$, $P < 0.01$) to determine measurement variation for fragment sizes between 2.0 and 21.0 kbp, with the regression being

$$(1) \qquad \ln(SD) = 2.384 + 0.000238(FS), \; r^2 = 0.94$$

We used a binning procedure (Gill et al. 1990; Galbraith et al. 1991) to assign an observed fragment to a particular molecular size range or bin, and used eq. 1 to estimate the size intervals or bin limits in SD. The 31 bins for *Rsa*I- and *Hin*fI-digested DNA probed with Ssa1 had the following limits: 2000–2100 bp (1), 2101–2200 bp (2), 2201–2300 bp (3), 2301–2400 bp (4), 2401–2500 bp (5), 2501–2600 bp (6), 2601–2700 bp (7), 2701–2800 bp (8), 2801–2900 bp (9), 2901–3100 bp (10), 3101–3300 bp (11), 3301–3500 bp (12), 3501–3700 bp (13), 3701–3900 bp (14), 3901–4100 bp (15), 4101–4400 bp (16), 4401–4700 bp (17), 4701–5000 bp (18), 5001–5500 bp (19), 5501–6000 bp (20), 6001–6500 bp (21), 6501–7000 bp (22), 7001–7700 bp (23), 7701–8500 bp (24), 8501–9300 bp (25), 9301 – 10 000 bp (26), 10 001 – 11 000 bp (27), 11 001 – 13 000 bp (28), 13 001 – 16 000 bp (29), 16 001 – 19 000 bp (30), 19 001 – 23 000 bp (31). When calculated at the midpoint of each bin, the average bin width was 7.7 SD for bins containing bands less than 3 kbp, 8.2 SD for bins containing bands 3–4 kbp, 7.8 SD for bands 4–7 kbp, and 7.1 SD for bands greater than 7 kbp. Average bin width over all 31 bins was 7.9 SD. Bins 6–24 were used to categorize the bands

observed for *Rsa*I-digested DNA probed with pSsa-A34, resulting in 19 observed alleles. Bins 1–22 were used to categorize the bands observed for the *Hin*fI-digested DNA probed with OtsPBS-1. A total of 115 bins was used to summarize band counts over all combinations of probes and restriction enzymes.

Data analysis

For each stock, mean band counts per fish (Ssa1, OtsPBS-1) or allele frequencies (pSsa-A34) were determined for each of the bins defined in the analysis. Annual variation in the distribution of band counts or allele frequencies was evaluated for each probe and restriction enzyme combination used for six populations that had been sampled for 2 or 3 years. The Kwinageese River stock was omitted from this evaluation owing to small total sample size. The χ^2 test with Monte Carlo simulations of the distribution of the χ^2 statistic (Roff and Bentzen 1989) was used to evaluate significance of annual variation in band count or allele frequency distributions, as well as differentiation among regional groupings of stocks. We used 100 simulations of the original data using the MONTE program of the REAP software package (McElroy et al. 1992).

Differences in band counts or allele frequencies were used to calculate a generalized squared distance value (D^2) (Pimentel 1979) between each pair of stocks. A dendrogram was obtained by clustering the distance estimates using the neighbour-joining algorithm (NEIGHBOR in PHYLIP) (Felsenstein 1990).

Individual fish were classified to specific stocks or regions using the DISCRIM procedure of SAS (SAS Institute Inc. 1989) with a jackknife sampling procedure. Classification functions were developed using all fish sampled except the one to be classified, with each fish tested individually in turn. This procedure evaluated the probability of successfully classifying unknown fish to specific stocks or regions using a 30-stock baseline (Teslin River deleted owing to small (<5) sample size).

Estimation of stock composition

Baseline data

To estimate the stock composition in samples from mixed stock fisheries, it is necessary to determine the probability of choosing a fish with a particular set of characteristics from a given population. The baseline data for each stock must be expressed as a conditional probability distribution. The maximum likelihood model used in our analysis (Fournier et al. 1984) requires that all characteristics used in the analysis be independent. The bands observed with Ssa1 or OtsPBS-1 were not all independent of each other. Accordingly, as outlined by Beacham et al. (1995), principal components analysis (PRINCOMP procedure using correlation matrix in SAS) was used to construct uncorrelated variables from the original 115 variables. In our analysis, the first 105 principal components accounted for 100% of the observed variation in the original 115 variables. Because the computer program of Fournier et al. (1984) required input as discrete frequency data for each baseline stock, the continuous frequency distributions of the principal component scores were represented as 12-bin histograms with limits of ≥2.50 (1), 2.49–1.50 (2), 1.49–1.00 (3), 0.00–0.60 (2), 0.59–0.30 (5), and 0.20–0.00 (6), with the limits all positive for the 6 bins greater than 0, and all negative for the 6 bins less than 0. Each stock was then characterized as an input matrix of 105 rows (principal components) and 12 columns (counts of principal component scores in each of the 12 bins).

Ssa1 data

In our study two restriction enzymes were used to examine variation at the Ssa1 locus, and it was necessary to evaluate whether the second restriction enzyme produced additional information that was useful for estimating stock composition. To do this, we estimated the stock composition of single-stock samples of Skeena River chinook salmon using Ssa1 band counts from *Hin*fI-digested DNA only, and from both the *Hin*fI- and *Rsa*I-digested DNA. For both data sets, a test sample of 100 fish was generated by sampling the appropriate Skeena River stock with replacement 50 times (stock composition estimated for each test sample) to compute estimates of the mean and standard deviation of the estimated stock contribution. Six Skeena River stocks were included in the baseline to resolve each sample, with each stock randomly resampled with replacement to obtain a new distribution of band counts with the same sample size (Millar 1990). The stock composition of each test sample was estimated as described by Fournier et al. (1984).

For the *Hin*fI-only data, 26 principal components summarized 100% of the observed variation from the original 31 bin frequency intervals for the six Skeena River chinook stocks. For the combined *Hin*fI and *Rsa*I data, 54 principal components incorporated 100% of the observed variation from the original 62 bins. The stock composition estimates generated from the two data sets were compared to determine if band count data from the two enzymes provided better estimates for the single-stock samples (true value = 100%) than the *Hin*fI band counts alone.

Utility of minisatellite data for stock composition estimation

The utility of the observed minisatellite variation was investigated initially by constructing a series of hypothetical fishery samples that contained fish from only a single major river drainage system (Fraser, Skeena rivers), with the stock composition estimated from a baseline comprised of stocks from that drainage system. As before, 100-fish test mixtures were generated 50 times by sampling the appropriate stocks with replacement, and the mean and SD of the estimated stock composition are reported. Stock compositions were first estimated under the assumption that observed band counts were perfectly representative of the stocks, demonstrating the potential utility of the method. To determine the present utility of the method, given the relatively small samples for many populations, we simulated random variation involved in the collection of the baseline samples. As before, each baseline stock was resampled with replacement for the estimation of the stock composition of each mixture to obtain new distributions of band counts of the same sample size as in the original one.

Results

Geographic variation in Ssa1 band counts

Substantial differentiation in band count distributions was observed between chinook salmon stocks from northern and southern British Columbia, among regional groups of stocks, and to some degree among stocks within regions. For *Rsa*I-restricted DNA, the mean number of bands per fish between 3.3 and 3.5 kbp (bin 12) in southern stocks was 0.82 for Fraser River, 0.94 for east coast of Vancouver Island (ECVI), 1.00 for west coast of Vancouver Island (WCVI), and 0.91 for southern mainland stocks, whereas it was 0.54 for northern mainland, 0.53 for Skeena River, 0.43 for Nass River, and 0.25 for Yukon River stocks.[2] Within smaller geographic areas, marked differences in band counts were also observed among stocks, with mean Fraser River band counts for bin 10 (0.05) lower than that of ECVI (0.59) or WCVI (0.49). Mean band count distributions of *Rsa*I-digested DNA were different between pooled WCVI and pooled ECVI stocks ($\chi^2 = 208.2$, df = 30, $P < 0.01$), between pooled ECVI and pooled Fraser

[2] Complete set of mean band counts per fish at 31 bin intervals for 31 stocks of chinook salmon whose genomic DNA was restricted with *Rsa*I and hybridized with the probe Ssa1 may be purchased (as Appendix 1) from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council of Canada, Ottawa, ON K1A 0S2, Canada.

River stocks ($\chi^2 = 471.3$, df = 30, $P < 0.01$), between pooled southern and pooled northern mainland stocks ($\chi^2 = 166.7$, df = 30, $P < 0.01$), but not between the pooled Skeena River stocks and the single Nass River stock (Kwinageese) sampled ($\chi^2 = 45.2$, df = 30, $P = 0.14$). Substantial variation was also observed among stocks within a single region. For example, in the Fraser River, the mean count of bin 18 in the lower Harrison River stock (0.03) was less than that of all upper river stocks (≥0.17).[2] In the Skeena River drainage, the Upper Kitsumkalum River flows into Kitsumkalum Lake, which is subsequently drained by the Lower Kitsumkalum River. Both the Upper and Lower Kitsumkalum rivers support spawning populations of chinook salmon. Substantial variation in band counts between these two populations was observed, with observed mean counts in 7 of the 31 bins differing by at least 0.10. Thus, the observed heterogeneity in band mean counts ranged from broad regional differentiation to differences between spawning populations within a tributary to a major river.

For *Hin*fI-restricted DNA, substantial variation in Ssa1 band distributions provided a pattern similar to that of *Rsa*I-restricted DNA. Large differences in mean band counts were generally observed between northern and southern chinook salmon stocks. For example, the mean number of bands in bin 15 was >0.10 in 10 of 12 northern stocks, but only 5 of 19 southern stocks.[3] Band count differences among smaller geographic regions were also observed, with mean count differences exceeding 0.20 for bins 10, 16, 19, and 25 between ECVI and WCVI stocks. As with *Rsa*I-digested DNA, regional differences in band count distributions were observed between pooled ECVI and WCVI stocks ($\chi^2 = 207.4$, df = 30, $P < 0.01$), between ECVI and Fraser River stocks ($\chi^2 = 222.6$, df = 30, $P < 0.01$), and between southern and northern mainland stocks ($\chi^2 = 154.1$, df = 30, $P < 0.01$), but not between the Skeena River and the single Nass River stock (Kwinageese) ($\chi^2 = 38.2$, $P = 0.10$). Differences in band counts among stocks within a single major river drainage were also observed (e.g., bins 25 and 28 for Harrison River versus other Fraser River stocks).[3] Differences in Ssa1 band counts provided both large-scale regional definition and fairly fine-scale differentiation among stocks in the tributaries of a major river drainage.

Geographic variation in pSsa-A34 allele frequencies

The pSsa-A34 probe detected bands between 2.5 and 8.5 kbp, a narrower molecular weight range than that of Ssa1 (Fig. 2). No alleles >8.5 kbp were ever observed. Unlike Ssa1, there were no consistent differences in pSsa-A34 allele frequencies between stocks in southern and northern regions.[4] However, there were substantial differences in allele frequencies among stocks in smaller geographic regions. For example, the mean frequency of the 4.1- to 4.4-kbp allele (bin 16) differed between WCVI (mean frequency 0.099) and ECVI stocks (0.288), with the entire distribution of allele frequencies significantly different between the two regional stock groupings ($\chi^2 = 276.8$, df = 18, $P < 0.01$). Similarly, regional differences in allele frequencies were observed between ECVI and Fraser River stocks ($\chi^2 = 191.6$, df = 18, $P < 0.01$) and between southern and northern mainland stocks ($\chi^2 = 75.2$, df = 18, $P < 0.01$), but not between the Skeena River and the single Nass River stock ($\chi^2 = 21.1$, df = 18, $P = 0.13$). Allele frequency differences were observed between some stocks within river drainages. In the upper Fraser River stocks, the frequency of the 3.5- to 3.7-kbp allele (bin 13) was 0.26 for the Stuart River and 0.09 for the Nechako River. For the six Skeena River stocks, the frequency of the 5.5- to 6.0-kbp allele (bin 20) ranged from 0.06 to 0.26. As with Ssa1, differences in Ssa-A34 allele frequencies were observed between chinook salmon stocks in fairly broad regional groups, as well as among stocks in localized areas of a river system. The standardized genetic variance (F_{ST}) among all stocks was 0.06, indicative that 6% of the observed genetic variation was due to allelic differentation among stocks, and about 94% of the variation was common to all stocks.

Heterozygosity at the Ssa-A34 locus was 83.9% over all chinook salmon sampled in the study. Stocks from the east coast of Vancouver Island (mean 78.5%, $n = 296$) and the Yukon River (mean 70.5%, $n = 78$) were less heterozygous than those from the west coast of Vancouver Island (87.9%, $n = 239$), the Skeena River (88.7%, $n = 292$), the Fraser River (81.2%, $n = 293$), or mainland British Columbia (88.3%, $n = 321$). In the Fraser River drainage, heterozygosity decreased with increasing distance of spawning migration. The lower river Harrison River stock was the most heterozygous (89.6%, $n = 96$), the long-migrating Tete Jaune stock was the least heterozygous (73.5%, $n = 49$), and the stocks between these two stocks were of intermediate heterozygosity (78.3%, $n = 148$).

Geographic variation in OtsPBS-1 band counts

As with the other probes used in this study, marked regional differentiation in the distribution of OtsPBS-1 band counts was observed. There was no consistent differentiation between northern and southern stocks,[5] but different distributions of band counts were observed between ECVI and WCVI stocks ($\chi^2 = 160.9$, df = 21, $P < 0.01$), between ECVI and Fraser River stocks ($\chi^2 = 321.2$, df = 21, $P < 0.01$), between southern and northern mainland stocks ($\chi^2 = 56.1$, df = 21, $P < 0.01$), and between the Skeena River stocks and the single Nass River stock ($\chi^2 = 37.0$, df = 21, $P = 0.01$). As with the other probes, differences in the distribution of OtsPBS-1 band counts were observed among stocks within a drainage system. For example, mean band counts per fish for the 3.5- to 3.7-kbp band in the Skeena River stocks varied from 0.23 to 0.71. Thus, the

[3] Complete set of mean band counts per fish at 31 bin intervals for 31 stocks of chinook salmon whose genomic DNA was restricted with *Hin*fI and hybridized with the probe Ssa1 may be purchased (as Appendix 2) from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council of Canada, Ottawa, ON K1A 0S2, Canada.

[4] Complete set of allele frequencies at 19 bin intervals for 31 stocks of chinook salmon whose genomic DNA was restricted with *Rsa*I and hybridized with the probe pSsa-A34 may be purchased (as Appendix 3) from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council of Canada, Ottawa, ON K1A 0S2, Canada.

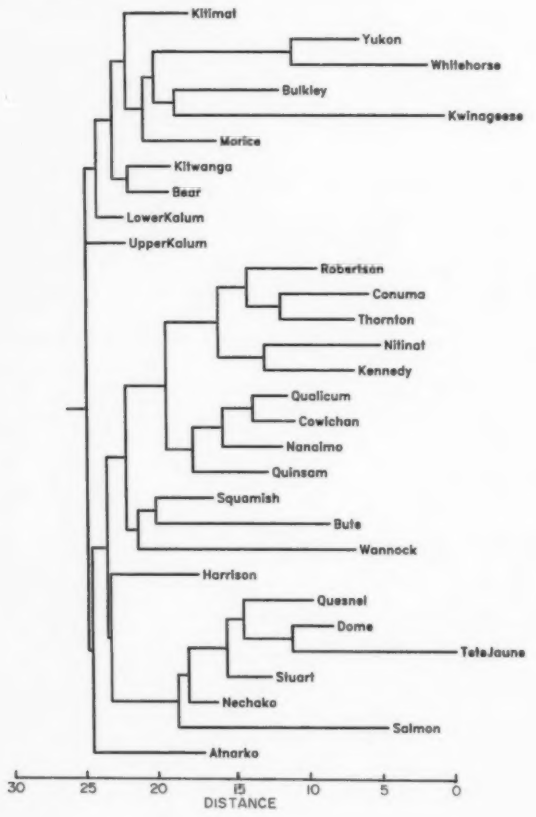
[5] Complete set of mean band counts per fish at 31 bin intervals for 31 stocks of chinook salmon whose genomic DNA was restricted with *Hin*fI and hybridized with the probe OtsPBS-1 may be purchased (as Appendix 4) from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council of Canada, Ottawa, ON K1A 0S2, Canada.

Table 2. Comparison of annual variation in band counts and allele frequencies for six populations of chinook salmon whose genomic DNA was restricted with *Rsa*I or *Hin*fI and hybridized with Ssa1, pSsa-A34, or OtsPBS-1.

		Ssa1–RsaI		Ssa1–*Hin*fI		pSsa-A34		OtsPBS-1	
Stock	Years	*N*	Prob.	*N*	Prob.	*N*	Prob.	*N*	Prob.
Quinsam	1987,1988,1992	41,23,44	0.39	41,23,44	0.53	43,23,42	0.36	40,21,42	0.07
Big Qualicum	1988,1992	49,36	0.60	49,35	0.88	47,37	0.67	49,39	0.33
Harrison	1988,1992	51,47	0.54	50,48	0.34	48,48	0.74	45,43	0.77
Nitinat	1988,1989	21,30	0.65	20,33	0.03	21,33	0.77	21,32	0.62
Stuart	1991,1992	17,8	0.27	22,8	0.28	22,9	0.47	26,8	0.78
Nechako	1991,1992	38,10	0.08	38,10	0.36	39,9	0.03	37,10	0.37

Note: The probability levels indicated are those derived from the χ^2 test with 100 Monte Carlo simulations of the distribution of the χ^2 statistic to estimate the probability of obtaining a value equal to or greater than that observed (Roff and Bentzen 1989). *N* is sample size in each year.

Fig. 3. Neighbour-joining dendrogram of relationships among 30 chinook salmon stocks based upon a generalized chord distance (Mahalanobis) calculated from allele frequencies and band counts for the restriction enzymes and probes indicated in Fig. 2. See Fig. 1 for the geographic locations of the individual stocks.



distribution of band counts again varied both among regions and among stocks within regions.

Annual variation in band counts and allele frequencies

Samples from three Vancouver Island stocks (Quinsam, Big Qualicum, and Nitinat) and three Fraser River stocks (Harrison, Stuart, Nechako) were obtained from two or three brood years. Of the individual tests conducted comparing the band count or allele frequency distributions for each probe-restriction enzyme combination, two of the tests were significant (Table 2). However, the significance level of the tests needed to be corrected to account for the simultaneous multiple tests conducted. When this was done as outlined by Lessios (1992), none of the comparisons was significant at the 5% probability level. This indicated that the distributions of band counts or allele frequencies were stable stock characteristics.

Stock structure

Neighbour-joining analysis of the distance matrix derived from band counts and allele frequencies indicated that there were two main groups of chinook salmon, with the southern stocks forming one group and the northern stocks the second group. The southern group was composed of stocks from the Fraser River, Vancouver Island, and the southern mainland (stocks 1–19 in Fig. 1), whereas the second group was composed of stocks from the northern mainland, the Skeena River, and the Yukon River (stocks 20–30 in Fig. 1) (Fig. 3). This split between southern and northern stocks was further illustrated by principal components analysis, in which along the axis of the first principal component most southern stocks were <0 and northern stocks >0 (Fig. 4). In the PCA, the Wannock River stock (19), the most northern stock of the southern group, was more similar to northern stocks than to southern ones, unlike the stock structure of Fig. 3. In the southern group, regional differentiation among the stocks was observed along the second principal component (Fig. 4). Vancouver Island stocks were distinct from Fraser River or mainland stocks. The northern mainland stocks (Kitimat, Atnarko) were split between the two regions in the neighbour-joining analysis (Fig. 3), but Atnarko River seemed more similar to northern stocks in the principal components analysis (Fig. 4). In the northern group, substantial variation was observed among the six Skeena River stocks, with the Bulkley River stock the most distinctive of them (Figs. 3, 4). The single Nass River stock (Kwinageese) was distinct from the six Skeena River stocks (Fig. 4).

Estimation of stock composition

Variation detected by Ssa1 is multibanded but under the control of a single locus, and in our study it was scored using two different restriction enzymes. To determine if additional stock

Fig. 4. Plot of the 30 stocks of chinook salmon along the first two principal components. Individual stocks are numbered as in Fig. 1.

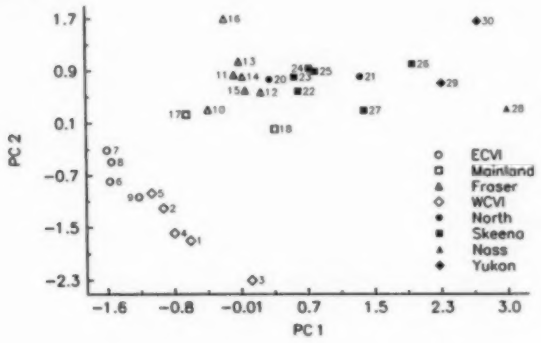


Table 3. Comparison of accuracy and precision of estimated stock compositions of single-stock (100%) samples of Skeena River drainage chinook salmon estimated using six baseline stocks.

	Method 1	Method 2
Bulkley	77.2±7.9	90.3±6.2
Kitwanga	67.4±8.5	90.8±5.4
Morice	73.7±9.5	89.2±5.3
Bear	67.7±9.6	86.4±7.8
Lower Kalum	64.1±8.2	88.7±6.4
Upper Kalum	73.4±8.2	91.3±5.6

Note: In method 1, band counts from genomic DNA restricted with *Hin*fI and hybridized with the probe Ssa1 were used in the estimation. In method 2, the data from method 1 were used in addition to the band counts from DNA restricted with *Rsa*I and hybridized with Ssa1. In each method, a 100-fish mixture was generated 50 times with replacement and the band distributions of the baseline populations were resampled. Values are given as the mean ± standard deviation derived from the 50 estimates of the percentage of the single population in the test sample.

separation information was derived from the *Rsa*I genomic DNA digests in addition to *Hin*fI-digested genomic DNA, we conducted preliminary analyses using the band count distributions of *Hin*fI alone and of *Hin*fI and *Rsa*I combined to characterize the six baseline stocks and single-stock samples of Skeena River chinook salmon. The average error of estimation for the single-stock mixtures over all six Skeena River stocks was 29.4% when only the distribution of band counts of *Hin*fI-restricted DNA was used in the analysis (Table 3). The addition of the distribution of band counts of *Rsa*I-restricted DNA from the same baseline stocks reduced the estimation error to 10.6% over all stocks, and increased the precision of the estimates. The use of both sets of band count distributions provided increased accuracy and precision in the estimation of stock composition, and the data from both *Hin*fI and *Rsa*I were used in subsequent analyses.

Distinguishing geographically close stocks such as those from different tributaries within a drainage system is a difficult task to accomplish using biological markers. We evaluated the utility of minisatellite DNA variation for this task in several ways. Stocks in two major river drainage systems (Fraser, Skeena) were surveyed to determine whether differences among stocks were sufficient to allow reliable estimation of stock composition. Estimates of stock composition were first determined under an assumption of exact determination of the band count distribution for each stock in the baseline with a resampling of the fishery sample, and secondly with a resampling of both the baseline and fishery samples. Simulated Fraser River fishery samples were developed to mimic times when upper river early-run fish predominated in the samples, and when lower river late-run Harrison River fish predominated. The accuracy of estimated stock composition under the fixed baseline band counts and allele frequencies was extremely high, with a 0% error rate observed in all stocks (examples 1–3, Table 4). With the baseline resampled, the mean error rate by stock ranged from 4.0% in example 1 to 1.3% in example 3. Very accurate estimates of stock composition can be obtained for samples from a lower Fraser River fishery. The accuracy of upper Fraser River stock composition estimates was extremely high, with a 0% error rate for a fixed band count and allele frequency in the baseline stocks, and mean error rates ranging from 0.1 to 0.5% when the baseline was resampled (examples 4–7, Table 4). Estimates of stock composition for all of the upper river stocks were quite accurate in all of the examples, with the Nechako and Stuart stocks well separated (the Stuart River flows into the Nechako River, which then enters the Fraser River).

In northern British Columbia, separation of Skeena River and Nass River chinook salmon is of management interest. Although only one Nass River stock (Kwinageese) was sampled, it was detected accurately in mixed stock samples of either Nass or Skeena River fish (examples 1–5, Table 5). Various mixtures of Skeena River stocks were estimated with 0% error using fixed band count and allele frequency data in the baseline stocks, and with 0.3 to 1.2% error using resampled baseline band counts and allele frequencies. The lower and upper Kitsumkalum River stocks, separated by run timing and Kitsumkalum Lake, were each quite distinct, as evidenced by the accurate composition estimate of the pure lower Kitsumkalum sample (example 5, Table 5). The additional use of the probes pSsa-A34 and OtsPBS-1 resulted in increased accuracy and precision of estimated stock composition of Skeena River mixtures over that which had previously been achieved using only Ssa1 (Tables 3 and 5).

The effect of mixture size on the accuracy and precision of estimated stock composition was investigated for a mixture of known proportions of Skeena River stocks. There was little effect on accuracy for mixtures of 25–100 fish, but the precision of the estimates increased with increasing mixture size (Table 6). In particular, stocks constituting only 8% of a mixture of 25 fish were represented by only 2 fish, yet accurate (but not overly precise) estimates of stock composition were obtained, even for those stocks for which the ability to identify individuals to specific stocks was poor (such as Kitwanga, see next section). Coefficients of variation for the Bulkley River contribution (32%) ranged from 9% for a 200-fish sample to 27% for a 25-fish sample (Table 6). Coefficients of variation for the upper Kitsumkalum River contribution (8%) ranged from 24% for a 200-fish sample to 65% for a 25-fish sample.

Identification of individuals

Some stock identification tasks require the classification of individuals to specific stocks. In the Fraser River drainage, the

Table 4. Estimated percent composition of a variety of mixtures of Fraser River chinook salmon.

Type	Harrison	Quesnel	Stuart	Nechako	Salmon	Dome	Tete Jaune
			Example 1				
True	0.0	10.0	20.0	20.0	10.0	20.0	20.0
Fixed	0.0±0.0	10.0±0.0	20.0±0.0	20.0±0.0	10.0±0.0	20.0±0.0	20.0±0.0
Resampled	12.0±6.7	8.8±2.2	17.1±3.7	18.5±4.2	8.4±2.0	18.0±4.3	17.2±3.3
			Example 2				
True	40.0	10.0	10.0	10.0	10.0	10.0	10.0
Fixed	40.0±0.0	10.0±0.0	10.0±0.0	10.0±0.0	10.0±0.0	10.0±0.0	10.0±0.0
Resampled	45.6±4.1	9.2±4.8	8.9±1.9	9.3±2.9	8.7±5.6	9.2±2.9	9.1±4.0
			Example 3				
True	70.0	10.0	10.0	10.0	0.0	0.0	0.0
Fixed	70.0±0.0	10.0±0.0	10.0±0.0	10.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Resampled	73.9±3.6	7.9±1.9	8.6±2.3	9.5±1.9	0.0±0.0	0.0±0.0	0.1±0.1
			Example 4				
True	—	10.0	20.0	20.0	10.0	20.0	20.0
Fixed	—	10.0±0.0	20.0±0.0	20.0±0.0	10.0±0.0	20.0±0.0	20.0±0.0
Resampled	—	9.7±2.4	19.4±3.8	20.8±4.1	10.0±2.9	20.6±4.3	19.6±4.1
			Example 5				
True	—	0.0	0.0	100.0	0.0	0.0	0.0
Fixed	—	0.0±0.0	0.0±0.0	100.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Resampled	—	0.0±0.0	0.0±0.0	99.3±2.3	0.0±0.0	0.0±0.0	0.7±2.2
			Example 6				
True	—	0.0	100.0	0.0	0.0	0.0	0.0
Fixed	—	0.0±0.0	100.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Resampled	—	0.0±0.0	98.2±3.6	0.9±2.7	0.0±0.0	0.0±0.0	0.9±2.7
			Example 7				
True	—	0.0	0.0	0.0	0.0	0.0±0.0	100.0
Fixed	—	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	100.0±0.0
Resampled	—	0.0±0.0	0.0±0.0	0.4±1.7	0.2±0.9	0.0±0.0	99.4±1.8

Note: Each mixture of 100 fish was generated 50 times with replacement, and the stock composition of the mixtures was estimated either using a fixed distribution of the band counts and allele frequencies in the baseline populations or by randomly resampling each baseline population with replacement to obtain a new distribution of band counts and allele frequencies for each baseline population, with the same sample size in the new distribution as in the original one. Band counts and allele frequencies were derived from genomic DNA restricted with *Rsa*I and hybridized with the probes Ssa1 and pSsa-A34, and DNA restricted with *Hin*fI and hybridized with Ssa1 and OtsPBS-1. Examples 1–3 included all seven populations in the baseline to simulate sampling in the lower Fraser River, whereas examples 4–7 included six baseline populations (Harrison excluded) to simulate upper Fraser River sampling. Values are given as the mean ± standard deviation.

lower Harrison River stock was generally distinct from upper river stocks (Fig. 3). When the seven Fraser stocks were used as a baseline, the highest accuracy of classification was achieved for Harrison River chinook salmon. About 76% of the Harrison River individuals tested were correctly classified even though the tested individuals were not used in the development of the discriminant functions (Table 7). Over all stocks, about 60% of the Fraser River fish were correctly classified, with the least accuracy obtained for the Salmon River fish, presumably owing to the small baseline sample size. Classification accuracy for Skeena River fish was generally lower than for Fraser River fish (Table 7). Bulkley River salmon, previously shown to be the most distinctive, were classified most accurately (60%). For the Fraser and Skeena river drainages, minisatellite variation improved the classification of individuals to specific stocks markedly over that expected by chance alone.

The classification of individuals to stock becomes more difficult as the number of stocks in the baseline increases and differentiation among stocks declines. An individual would be correctly classified to one of 30 stocks about 3.3% of the time by chance alone. Individual classification to specific stocks using a 30-stock baseline ranged from 6% correct in Kitwanga to nearly 70% correct for the mainstem Yukon River (Table 8), better than that expected by chance. Classification to region of origin (outlined in Fig. 1) was substantially more accurate than that to individual stock. For example, only 20% of Quesnel River fish were correctly identified to the Quesnel River, but 80% were correctly identified to the Fraser River drainage (Table 8). Classification accuracy to region of origin ranged from 9% for Nass River (Kwinageese) to over 80% for Fraser River chinook salmon (Table 8). Although improvements can clearly still be made, the minisatellite DNA variation detailed in our study provides a basis to identify individuals to a specific stock or region of origin.

Discussion

The three probes used in this study detected sufficient genetic variation at a limited number of loci in chinook salmon to provide strong regional stock structure and accurate stock composition estimation in simulated mixed-stock fishery samples. Each of the pSsa-A34 and Ssa1 probes hybridize with chinook salmon DNA fragments arising from a single locus,

Table 5. Estimated percent composition of 100-fish mixtures of Skeena River and Nass River (Kwinageese) chinook salmon.

Type	Bulkley	Kitwanga	Morice	Bear	L. Kalum	U. Kalum	Kwinageese
			Example 1				
True	0.0	0.0	0.0	0.0	0.0	0.0	100.0
Fixed	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	100.0±0.0
Resampled	0.6±2.7	1.7±4.7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	97.6±5.4
			Example 2				
True	25.0	25.0	15.0	15.0	10.0	10.0	0.0
Fixed	25.0±0.0	25.0±0.0	15.0±0.0	15.0±0.0	10.0±0.0	10.0±0.0	0.0±0.0
Resampled	24.4±3.1	24.0±3.7	15.0±2.9	15.0±3.2	10.7±2.9	10.8±3.5	0.0±0.0
			Example 3				
True	30.0	5.0	5.0	30.0	25.0	5.0	0.0
Fixed	30.0±0.0	5.0±0.0	5.0±0.0	30.0±0.0	25.0±0.0	5.0±0.0	0.0±0.0
Resampled	28.4±4.5	6.2±2.4	5.5±2.0	28.0±4.2	25.0±3.6	6.8±3.2	0.0±0.0
			Example 4				
True	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Fixed	100.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Resampled	98.1±2.8	0.3±1.1	0.2±0.7	0.0±0.0	0.9±1.2	0.5±1.5	0.0±0.0
			Example 5				
True	0.0	0.0	0.0	0.0	100.0	0.0	0.0
Fixed	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	100.0±0.0	0.0±0.0	0.0±0.0
Resampled	0.2±1.2	0.3±1.0	0.2±1.0	0.0±0.0	98.5±2.4	0.8±1.7	0.0±0.0

Note: Data and methods used are outlined in Table 4. All seven stocks were included in the baseline used to estimate the stock composition of the mixtures. Values are given as the mean ± standard deviation.

Table 6. Evaluation of mixture size on accuracy and precision of estimated stock composition of mixtures containing Skeena River chinook salmon.

	Mixture size (No. of fish)						
	True	200	150	100	75	50	25
Bulkley	32.0	31.4±2.7	31.9±3.7	30.0±4.2	31.0±4.0	31.1±6.2	31.6±8.7
Kitwanga	8.0	8.4±2.1	8.0±2.6	8.3±2.6	7.1±2.9	8.8±3.7	9.6±6.0
Morice	20.0	20.3±2.7	20.5±4.1	20.5±4.2	20.8±4.3	20.9±5.5	17.6±7.5
Bear	8.0	7.9±1.9	7.9±2.2	8.3±2.6	8.3±3.6	7.6±3.9	7.9±4.5
Lower Kalum	24.0	23.9±2.6	23.4±3.5	24.5±4.3	23.8±5.2	22.6±6.4	22.8±9.3
Upper Kalum	8.0	7.8±1.9	7.8±2.0	7.9±2.6	8.2±3.7	8.2±3.9	9.3±6.0
Other stocks	0.0	0.3±0.5	0.4±0.8	0.5±0.7	0.7	0.8±1.2	1.2±2.2
Mean error		0.3	0.3	0.6	0.6	0.8	1.2
Mean coefficient of variation		0.18	0.22	0.25	0.31	0.36	0.49

Note: Mixture sizes of 200, 150, 100, 75, 50, and 25 fish were generated with replacement from an original mixture containing the specified proportions of six Skeena River stocks. A 30-stock baseline with a fixed distribution of band counts and allele frequencies was used to estimate stock compositions. Values are given as the mean ± standard deviation derived from 50 estimates of the percent contribution of each stock in the mixture.

as is the case for Ssa1 in Atlantic salmon (Bentzen et al. 1993), sockeye salmon (Taylor et al. 1996), and coho salmon (Miller et al. 1996), and for pSsa-A34 in Atlantic salmon (*Salmo salar*) (Taggart et al. 1995), brown trout (*Salmo trutta*) (Prodöhl et al. 1994), and sockeye salmon (Taylor et al. 1996). The OtsPBS-1 probe hybridizes with DNA fragments representing one or both alleles from at least two loci in chinook salmon.

The structure of the Ssa1 locus in chinook salmon is apparently similar to that described for coho salmon (Miller et al. 1996). The alleles consist of tandemly duplicated copies of the original Ssa1 locus plus flanking regions containing restriction sites. Thus, in restricted DNA, each allele is represented by up to six DNA fragments that are cotransmitted to progeny. The different alleles consist of various combinations of shared restriction fragment subunits, but the same alleles do not share fragments over all restriction enzymes. The additional stock identification ability achieved in this study by the addition of the Ssa1 band count data from a second restriction enzyme may arise from two factors. Miller et al. (1996) found that some coho salmon Ssa1 alleles very similar in size and structure could be differentiated in the restriction patterns of some, but not all, enzymes. In addition, the observed differences among stocks are based on band, not allele, frequencies. Thus, two stocks characterized by different Ssa1 alleles will be best distinguished using band count data from a restriction enzyme for which the two alleles do not share any restriction fragments.

Stock identification of Pacific salmon is a complex problem, given the large number of stocks that can contribute to a fishery. In British Columbia, there are about 300 stocks of chinook salmon (Healey 1982), and this makes it logistically

Table 7. Percent correct classification of individual fish using discriminant analyses for stocks in the Fraser River and Skeena River drainages.

Stock	*N*	% correct
Fraser River		
Harrison	83	75.9
Quesnel	20	30.0
Stuart	22	40.9
Nechako	47	46.8
Dome	36	55.7
Salmon	6	33.3
Tete Jaune	48	68.8
Mean	262	59.2
Skeena River		
Bulkley	47	59.6
Kitwanga	52	17.3
Morice	45	40.0
Bear	32	25.0
Lower Kalum	47	27.7
Upper Kalum	48	35.4
Mean	271	34.3

Note: Only stocks located in the drainages were used to derive the discriminant functions, and results are indicated for a jackknife sample, as in Table 8. *N* is the number of fish classified in each stock.

and financially impractical to obtain representative samples from them all. Management of fisheries is usually based upon stock aggregates, rather than individual stocks, so the problem of obtaining representative baseline samples is reduced if stocks in localized areas are genetically more similar to each other than to stocks in more distant areas. In this case, the portion of the catch derived from unsampled stocks will be allocated to similar stocks in the baseline. The minisatellite DNA variation observed in our study indicated that there was regional structure of the chinook salmon stocks, with distinct groups of stocks from the Fraser River, ECVI, WCVI, the southern mainland, and northern British Columbia. Regional similarity among stocks will preclude the necessity of sampling all stocks potentially contributing to a fishery.

After examining variation at 25 polymorphic loci using protein electrophoresis, Utter et al. (1989) reported that chinook salmon stocks in the upper Fraser River drainage formed a distinct group, as did ECVI and southern mainland stocks, and WCVI stocks were similar to coastal stocks in Washington and Oregon. Our results indicated that ECVI stocks and southern mainland stocks were quite distinct, with ECVI stocks more similar to WCVI stocks than to mainland stocks. The protein electrophoretic study included a limited number of stocks from British Columbia, and the larger number of southern British Columbia stocks sampled in our study coupled with the genetic variation detected in minisatellite DNA suggest that ECVI and mainland chinook salmon stocks are genetically distinct.

A maximum likelihood method with a fixed or exact distribution of the baseline data has been commonly used to estimate the stock composition of fishery samples (Grant et al. 1980; Fournier et al. 1984; Beacham et al. 1985; Milner et al. 1985; Wood et al. 1989; Brodziak et al. 1992; Brodziak 1993). In the present study, estimates of stock composition were less accurate and precise when the baseline stocks were resampled to develop new distributions of band counts and allele frequencies. As the majority of the stocks had complete data sets from less than 50 fish, the difference between the estimates based on fixed and resampled baseline data indicates that sample size should be increased for many of the baseline stocks prior to application in fisheries management. Estimates of stock composition for stocks in either very high (e.g., 100%) or very low (0%) proportions usually have the greatest bias (Beacham et al. 1985; Wood et al. 1987). In our study, samples containing high (single stock) or low (<10%) abundance stocks were estimated with virtually no error. Similar results have been observed in the application of minisatellite DNA variation to stock composition estimation in coho salmon (Miller et al. 1996).

The simulations conducted for mixtures of either Fraser River or Skeena River chinook salmon stocks suggested that minisatellite DNA variation may be used to provide accurate and precise estimates of individual stocks in the mixtures. However, the applicability of minisatellite DNA variation to practical fishery management issues needs to be verified by applications in field situations, where fish in the mixtures can originate from stocks not in the baseline.

Protein electrophoresis has been applied extensively to describe genetic differentiation among chinook salmon populations (Gharrett et al. 1987; Winans 1989; Gall et al. 1992; Utter et al. 1992) and estimate stock compositions in chinook salmon fisheries (Utter et al. 1987; Waples and Aebersold 1990; Shaklee et al. 1990). However, estimates of stock composition are usually only available for chinook salmon from geographic areas (Utter et al. 1987; Marshall et al. 1991) and require large samples to obtain precise estimates of stock composition. For example, although 14 loci were scored, the coefficient of variation (CV) for some stocks contributing 8% of a 300-fish sample was in excess of 60%, and the CV of a stock constituting about 20% of a 900-fish sample was over 20% (Shaklee et al. 1990). In our analysis, the CV of a stock constituting 8% of a 200-fish mixture was about 24%, and the CV of a stock constituting 20–24% of the 200-fish sample was about 12%. Simulation analysis can provide a good indication of the precision that can be achieved in actual fishery applications. For example, the SD of a stock constituting <10% of a 200-fish mixture from a chum salmon fishery was about 5% (Beacham et al. 1987), comparable with that observed (5–6%) from an extensive set of simulated 200-fish mixtures of chum salmon (Anonymous 1992).

Galvin et al. (1995) compared the accuracy and precision of estimated stock compositions for mixtures of Atlantic salmon from the River Shannon in Ireland where data from eight allozyme loci and three minisatellite loci were available from nine tributary populations. They reported that mixtures incorporating just the minisatellite loci were about only 20% of the size required for mixtures incorporating just the allozyme loci to achieve any given level of precision. Although there are a number of factors to be considered in comparing results between actual fishery applications and simulated mixtures (such as individuals in the fishery sample from stocks not in the baseline, different numbers of stocks in the baseline), the results of simulations in our study and those of Galvin et al. (1995) suggest that with respect to protein electrophoresis, minisatellite DNA variation may offer the ability to accurately

Table 8. Percent correct classification of individual fish using discriminant analysis for 30 stocks of chinook salmon.

Stock	*N*	Correct	Fraser	ECVI	WCVI	Main (south)	Main (north)	Skeena	Nass	Yukon	Regional
Harrison	83	37.4	7.2	13.3	9.6	6.0	7.2	19.3	0.0	0.0	44.6
Quesnel	20	20.0	60.0	5.0	0.0	5.0	0.0	5.0	0.0	5.0	80.0
Stuart	22	31.8	50.0	4.6	0.0	9.1	0.0	4.6	0.0	0.0	81.8
Nechako	47	23.4	46.8	2.1	8.5	0.0	2.1	12.8	2.1	2.1	70.2
Dome	36	41.7	50.0	2.8	0.0	2.8	0.0	2.8	0.0	0.0	91.7
Salmon	6	33.3	66.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
Tete Jaune	48	66.7	31.3	0.0	0.0	0.0	0.0	2.1	0.0	0.0	98.0
Fraser	262	36.3	44.6	4.0	2.6	3.3	1.3	6.7	0.3	1.0	80.9
Big Qualicum	82	41.5	9.8	32.9	4.9	4.9	0.0	6.1	0.0	0.0	74.4
Quinsam	103	52.4	3.9	14.6	8.7	3.9	6.8	7.8	1.0	1.0	67.0
Nanaimo	44	29.6	4.5	38.6	9.1	9.1	0.0	9.1	0.0	0.0	68.2
Cowichan	50	30.0	6.0	46.0	2.0	4.0	4.0	8.0	0.0	0.0	76.0
ECVI	279	38.4	6.1	33.0	6.2	5.5	2.7	7.8	0.3	0.0	71.4
Conuma	46	56.5	6.5	2.2	30.5	0.0	0.0	2.2	2.2	0.0	87.0
Robertson	38	42.1	5.3	23.7	26.3	2.6	0.0	0.0	0.0	0.0	68.4
Thornton	36	27.8	2.8	13.9	41.7	8.3	0.0	2.8	0.0	2.8	69.5
Kennedy	42	42.9	7.1	14.3	33.3	2.4	0.0	0.0	0.0	0.0	76.2
Nitinat	49	57.2	8.2	2.0	18.4	8.2	0.0	6.1	0.0	0.0	75.6
WCVI	211	45.3	6.0	11.2	30.0	4.3	0.0	2.2	0.4	0.6	75.3
Squamish	87	41.4	10.3	19.6	6.9	4.7	4.6	12.6	0.0	0.0	46.1
Bute	55	38.2	12.7	1.8	10.9	23.6	3.7	5.5	3.6	0.0	61.8
Wannock	50	56.0	14.0	4.0	8.0	10.0	0.0	8.0	0.0	0.0	66.0
Main (south)	192	45.2	12.3	8.5	8.6	12.8	2.8	8.7	1.2	0.0	58.0
Atnarko	54	35.2	11.1	3.7	5.6	7.5	5.5	22.2	0.0	9.3	40.7
Kitimat	53	13.2	5.7	5.7	3.8	1.9	9.4	47.2	0.0	13.2	22.6
Main (north)	107	24.2	8.4	4.7	4.7	4.7	7.5	34.7	0.0	11.3	31.7
Bulkley	47	46.8	10.7	0.0	2.1	0.0	6.4	17.0	17.0	0.0	63.8
Kitwanga	52	5.8	17.3	3.9	1.9	1.9	3.9	53.9	5.8	5.8	59.7
Morice	45	31.1	6.7	6.7	0.0	2.2	6.7	40.0	4.4	2.2	71.1
Bear	32	18.8	12.5	6.3	3.1	9.4	3.1	40.6	6.3	0.0	59.4
Lower Kalum	47	12.8	19.2	4.3	8.5	4.2	10.7	31.9	0.0	8.5	44.7
Upper Kalum	48	6.3	25.0	4.2	8.3	12.5	10.4	20.8	4.2	8.3	27.1
Skeena	271	20.3	15.2	4.2	4.0	3.6	8.3	34.0	6.3	4.1	54.3
Nass	11	9.1	0.0	0.0	9.1	9.1	18.2	45.5	—	9.1	9.1
Yukon mainstem	17	11.8	5.9	0.0	0.0	0.0	5.9	29.4	5.9	41.2	53.0
Whitehorse	43	69.8	0.0	0.0	0.0	0.0	4.7	11.6	4.7	9.3	79.1
Yukon	60	40.8	3.0	0.0	0.0	0.0	5.3	20.5	5.3	25.3	66.1

Note: The percentage of fish that were correctly classified to stock is indicated, as is the percentage of fish classified to other stocks in the same and other regions. Results listed are for a jackknife sample, in which the fish tested was not included in the sample used to derive the discriminant functions. The regional percent correct is the sum of the percentage of fish correctly classified to stock and the percentage classified to other stocks within the same region. Stocks comprising each region are indicated in the table. *N* is the number of fish classified in each stock.

and precisely estimate stock composition on a much finer geographic scale, and to sample fewer fish to provide estimates of comparable precision.

An extensive program of marking chinook and coho salmon with coded-wire tags is currently in place in the Pacific northwest, with the tags applied primarily to hatchery-produced fish. One of the main uses of the coded-wire tag program is stock identification, in which the numbers of fish from particular stocks and stock groups caught in a particular fishery are estimated. This procedure requires examining a portion of the catch to identify marked adults (adipose fin removed), recovering tags from marked fish and decoding them, and expanding the number of tags recovered from each stock to account for unsampled catch, the proportion of the stock that was untagged, any lost tags, and tagged fish missed in the sample. Coded-wire tags have been used to estimate stock composition of chinook and coho salmon catches (Shaul and Clark 1990), but there can be substantial limitations to the accuracy and precision of the estimates. For example, in a simple three-stock mixture of coho salmon, and with 48% of the catch sampled for marks, Shaul and Clark (1990) concluded that the observed variation in the estimates did not allow for a definitive catch estimate by stock. Geiger (1990) outlined an example in which 50% of a stock was marked, 20% of the catch was sampled, and the stock constituted 10% of the catch, but the CV of the estimated composition was about 27%. However, a marking rate of 50% for a stock is unusually high, and a more realistic estimate of 5% of the

stock marked would result in a CV of about 100% in the example. The stock composition of catch estimated with minisatellite DNA variation outlined in our study may be more precise than that estimated using coded-wire tags, but the accuracy and precision of the stock composition derived from minisatellite DNA variation needs to be verified in the field.

Minisatellite DNA variation surveyed in our study may be an effective biological tag for use in estimation of stock composition. It has the advantages that all fish in a stock are naturally marked at no cost, and in the short term at least, the distributions of band counts and allele frequencies by stock were annually stable. Annual variability in some biological markers, such as scale pattern characteristics, can preclude their use for stock identification when representative reference samples cannot be obtained annually (Wood et al. 1989). In contrast, for minisatellite genetic data, once sufficient fish from a stock have been sampled to provide a representative distribution of the band counts and allele frequencies, it may be necessary only to sample fish from commercial or recreational fisheries to provide reliable estimates of stock composition.

Our analysis has indicated that minisatellite DNA variation has the potential to allow identification of individual fish to a specific stock or region of origin. In our survey, the use of three minisatellite probes coupled with a jackknife classification procedure resulted in a classification accuracy of about 35% to individual stock and of about 65% to region of origin for a 30-stock baseline. Classification accuracy increased when the possible stocks of origin were confined to a limited geographic area. In a river drainage, relatively accurate estimates of stock composition were obtained, but the accuracy of identifying individual fish to specific stocks was limited. For example, in the Skeena River drainage, the percentage of Kitwanga River chinook in the simulated mixtures could be accurately identified for percentages between 0 and 25%, but only 17% of Kitwanga River individuals could be correctly assigned to stock of origin. Estimation of the stock composition of a mixture and classification of individual fish are separate problems. The data from only the single fish to be identified are considered in classification problems, but in the estimation of stock composition, the data from all fish in the mixture are considered as a group. Higher levels of accuracy have been previously achieved in the estimation of stock composition using the entire mixture sample than in the individual classification of fish from the same mixture (Fournier et al. 1984).

Substantial potential has been suggested to exist in applying DNA variation to fisheries management (Hallerman and Beckman 1988), but applications to date have been limited. We examined variation in minisatellite DNA with the expectation that genomic variation would provide the means to discriminate among local stocks. Our results indicate that minisatellite DNA variation may be very useful for stock identification of chinook salmon within a drainage basin. Miller et al. (1996) demonstrated that minisatellite DNA variation in coho salmon (*Oncorhynchus kisutch*) also provided extremely accurate and precise estimates of stock composition in simulated fishery samples. The ability to estimate chinook salmon stock compositions of fishery samples can obviously be improved by using more loci, adding more stocks to the baseline, and increasing the number of fish sampled for each stock. Additional resolution may be provided by evaluating variation at microsatellite loci as an enhancement or alternative to existing stock identification techniques.

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References

Anonymous. 1992. Accuracy and precision of genetic stock identification for estimating the stock composition of mixed-stock chum salmon fisheries in northern Puget Sound and southern Georgia Strait. Report No. 92-2. Pacific Salmon Commission Joint Chum Salmon Technical Committee. Pacific Salmon Commission, Vancouver, B.C.

Beacham, T.D., Withler, R.E., and Gould, A.P. 1985. Biochemical genetic stock identification of chum salmon (*Oncorhynchus keta*) in southern British Columbia. Can. J. Fish. Aquat. Sci. **42**: 437–448.

Beacham, T.D., Gould, A.P., Withler, R.E., Murray, C.B., and Barner, L.W. 1987. Biochemical genetic survey and stock identification of chum salmon (*Oncorhynchus keta*) in British Columbia. Can. J. Fish. Aquat. Sci. **44**: 1702–1713.

Beacham, T.D., Murray, C.B., and Withler, R.E. 1989. Age, morphology, and biochemical genetic variation of Yukon River chinook salmon. Trans. Am. Fish. Soc. **118**: 46–63.

Beacham, T.D., Withler, R.E., and Wood, C.C. 1995. Stock identification of sockeye salmon using minisatellite DNA variation. N. Am. J. Fish. Manage. **15**: 249–265.

Bentzen, P., and Wright, J.M. 1993. Nucleotide sequence and evolutionary conservation of a minisatellite variable number tandem repeat cloned from Atlantic salmon, *Salmo salar*. Genome, **36**: 271–277.

Bentzen, P., Taylor, E.B., and Wright, J.M. 1993. A novel synthetic probe for DNA fingerprinting salmonid fishes. J. Fish Biol. **43**: 313–316.

Bermingham, E., Forbes, S.H., Friedland, K., and Pla, C. 1991. Discrimination between Atlantic salmon (*Salmo salar*) of North America and European origin using restriction analysis of mitochondrial DNA. Can. J. Fish. Aquat. Sci. **48**: 884–893.

Bernatchez, L., Guyomard, R., and Bonhomme, F. 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. Mol. Ecol. **1**: 161–173.

Brodziak, J. 1993. An extension of stock composition analysis to include marking data. Can. J. Fish. Aquat. Sci. **50**: 251–257.

Brodziak, J., Bentley, B., Bartley, D., Gall, G.A.E., Gomulkiewwicz, R., and Mangel, M. 1992. Tests of genetic stock identification using coded wire tagged fish. Can. J. Fish. Aquat. Sci. **49**: 1507–1517.

Bronstein, I., and McGrath, P. 1989. Chemiluminescence lights up. Nature (London), **338**: 599–600.

Bronstein, I., Voyta, J.C., Lazzari, K.A., Murphy, O., Edwards, B., and Kricka, L.J. 1990. Rapid and sensitive detection of DNA in

Southern blots with chemiluminescence. BioTechniques, **8**: 310–314

Feinberg, A.P., and Vogelstein, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**: 6–13.

Felsenstein, J. 1990. PHYLIP manual version 3.3. University Herbarium, University of California, Berkeley, Calif.

Fournier, D.A., Beacham, T.D., Riddell, B.E., and Busack, C.A. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. Aquat. Sci. **41**: 400–408.

Galbraith, D.A., Boag, P.T., Gibbs, H.L., and White, B.N. 1991. Sizing bands on autoradiograms: a study of precision for scoring DNA fragments. Electrophoresis, **12**: 210–220.

Gall, G.A.E., Bartley, D., Bentley, B., Brodziak, J., Gomulkiewicz, R., and Mangel, M. 1992. Geographic variation in population genetic structure of chinook salmon from California and Oregon. Fish. Bull. **90**: 77–100.

Galvin, P., McKinnell, S., Taggart, J.B., Ferguson, A., O'Farrell, M., and Cross, T.F. 1995. Genetic stock identification of Atlantic slamon (*Salmo salar* L.) using single locus minisatellite DNA profiles. J. Fish. Biol. **47**(Suppl. A): 186–199.

Geiger, H.J. 1990. Parametric bootstrap confidence intervals for estimating contributions to fisheries from marked salmon populations. Am. Fish. Soc. Symp. **7**: 667–676.

Gharrett, A.J., Shirley, S.M., and Tromble, G.R. 1987. Genetic relationships among populations of Alaskan chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. **44**: 765–774.

Gill, P., Sullivan, K., and Werrett, D.J. 1990. The analysis of hypervariable DNA profiles: problems associated with the objective determination of the probability of a match. Hum. Genet. **85**: 75–79.

Grant, W.S., Milner, G.B., Krasnowski, P., and Utter, F.M. 1980. Use of biochemical genetic variants for identification of sockeye salmon (*Oncorhynchus nerka*) in Cook Inlet, Alaska. Can. J. Fish. Aquat. Sci. **37**: 1236–1247.

Grewe, P.M., and Hebert, P.D.N. 1988. Mitochondrial DNA diversity among broodstocks of the lake trout, *Salvelinus namaycush*. Can. J. Fish. Aquat. Sci. **45**: 2114–2122.

Hallerman, E.M., and Beckmann, J.S. 1988. DNA-level polymorphism as a tool in fisheries science. Can. J. Fish. Aquat. Sci. **45**: 1075–1087.

Healey, M.C. 1982. Multispecies, multistock aspects of Pacific salmon management. *In* Multispecies approach to fisheries management advice. *Edited by* M.C. Mercer. Can. Spec. Publ. Fish. Aquat. Sci. No. 59. pp. 119–126.

Heath, D.D., Bernier, N.J., and Mousseau, T.A. 1995. A single-locus minisatellite discriminates chinook salmon (*Oncorhynchus tshawytscha*) populations. Mol. Ecol. **4**: 389–393.

Höltke, H.J., Sagner, G., Kessler, C., and Schmitz, G. 1992. Sensitive chemiluminescent detection of digoxigenin-labelled nucleic acids: a fast and simple protocol and its applications. BioTechniques, **12**: 104–112.

Jarman, A.P., and Wells, R.A. 1989. Hypervariable minisatellites: recombinators or innocent bystanders. Trends Genet. **5**: 367–371.

Jefferts, K.B., Bergman, P.K., and Fiscus, H.F. 1963. A coded-wire identification system for macro-organisms. Nature (London), **198**: 460–462.

Kornfield, I., and Bogdanowicz, S.M. 1987. Differentiation of mitochondrial DNA in Atlantic herring, *Clupea harengus*. Fish. Bull. **85**: 561–568.

Lessios, H.H. 1992. Testing electrophoretic data for agreement with Hardy–Weinberg expectations. Mar. Biol. (Berlin), **112**: 517–523.

Marshall, A.R., Miller, M., Busack, C., and Phelps, S.R. 1991. Genetic stock identification analysis of three 1990 Washington ocean and Strait of Juan de Fuca chinook salmon fisheries. GSI Summary Rep. No. 91–1. Washington Department of Fisheries, Olympia, Wash.

McElroy, D., Moran, P., Bermingham, E., and Kornfield, I. 1992. REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. J. Hered. **83**: 157–158.

Millar, R.B. 1990. Comparison of methods for estimating mixed stock fishery composition. Can. J. Fish. Aquat. Sci. **47**: 2235–2241.

Miller, K.M., Withler, R.E., and Beacham, T.D. 1996. Stock identification of coho salmon (*Oncorhynchus kisutch*) using minisatellite DNA variation. Can. J. Fish. Aquat. Sci. **53**: 181–195.

Milner, G.B., Teel, D.J., Utter, F.M., and Burley, C.L. 1981. Columbia River stock identification study: validation of the genetic method. National Oceanic and Atmospheric Administration, Northwest and Alaska Fisheries Center, Seattle, WA 98112.

Milner, G.B., Teel, D.J., Utter, F.M., and Winans, G.A. 1985. A genetic method of stock identification in mixed populations of Pacific salmon, *Oncorhynchus* spp. Mar. Fish. Rev. **47**: 1–8.

Noppinger, K., Duncan, G., Ferraro, D., Watson, S., and Ban, J. 1992. Evaluation of DNA probe removal from nylon membrane. BioTechniques, **13**: 572–575.

Palva, T.K., Lehvaslaiho, H., and Palva, E.T. 1989. Identification of anadromous and non-anadromous salmon stocks in Finland by mitochondrial DNA analysis. Aquaculture, **81**: 237–244.

Pimentel, R.A. 1979. Morphometrics: the multivariate analysis of biological data. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Prodöhl, P.A., Taggart, J.B., and Ferguson, A. 1994. Single locus inheritance and joint segregation analysis of minisatellite (VNTR) DNA loci in brown trout (*Salmo trutta* L.). Heredity, **73**: 556–566.

Ricker, W.E. 1972. Heredity and environmental factors affecting certain salmonid populations. *In* The stock concept of Pacific salmon. H.R. MacMillan lectures in fisheries. University of British Columbia, Vancouver, B.C. pp. 19–60.

Roff, D.A., and Bentzen, P. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small sample sizes. Mol. Biol. Evol. **6**: 539–545.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

SAS Institute Inc. 1989. SAS/STAT users guide. Version 6. 4th ed. Vol. 1. SAS Institute, Cary, N.C.

Shaklee, J.B., Busack, C., Marshall, A., Miller, M., and Phelps, S.R. 1990. The electrophoretic analysis of mixed-stock fisheries of Pacific salmon. *In* Isozymes: structure, function, and use in biology and medicine. *Edited by* Z.-I. Ogita and C.L. Markert. Progr. Clin. Biol. Res. **344**: 235–262.

Shaul, L.D., and Clark, J.E. 1990. Use of coded wire tag data to estimate aggregate stock composition of salmon catches in multiple mixed-stock fisheries. Am. Fish. Soc. Symp. **7**: 613–622.

Stevens, T.A., Withler, R.E., Goh, S.H., and Beacham, T.D. 1993. A new multilocus probe for DNA fingerprinting in chinook salmon (*Oncorhynchus tshawytscha*), and comparisons with a single-locus probe. Can. J. Fish. Aquat. Sci. **50**: 1559–1567.

Taggart, J.B., and Ferguson, A. 1990. Hypervariable minisatellite DNA single locus probes for the Atlantic salmon, *Salmo salar* L. J. Fish Biol. **37**: 991–993.

Taggart, J.B., Prodöhl, P.A., and Ferguson, A. 1995. Genetic markers for Atlantic salmon (*Salmo salar* L.): single locus inheritance and joint segregation analyses of minisatellite (VNTR) DNA loci. Anim. Genet. **26**: 13–20.

Taylor, E.B. 1995. Genetic variation at minisatellite DNA loci among North Pacific populations of steelhead and rainbow trout (*Oncorhynchus mykiss*). J. Hered. **86**: 354–363.

Taylor, E.B., Beacham, T.D., and Kaeriyama, M. 1994. Population structure and identification of North Pacific Ocean chum salmon (*Oncorhynchus keta*) revealed by an analysis of minisatellite DNA variation. Can. J. Fish. Aquat. Sci. **51**: 1430–1442.

Taylor, E.B., Foote, C.J., and Wood, C.C. 1996. Molecular genetic evidence for parallel life history evolution within a Pacific salmon (sockeye salmon and kokanee, *Oncorhynchus nerka*). Evolution, **50**: 401–416.

Utter, F.M., Teel, D.J., Milner, G.B., and McIsaac, D. 1987. Genetic estimates of stock compositions of 1983 chinook salmon harvests off the Washington coast and the Columbia River. Fish. Bull. **85**: 13–23.

Utter, F.M., Milner, G., Stahl, G., and Teel, D. 1989. Genetic population structure of chinook salmon, *Oncorhynchus tshawytscha*, in the Pacific northwest. Fish. Bull. **87**: 239–264.

Utter, F.M., Waples, R.S., and Teel, D.J. 1992. Genetic isolation of previously indistinguishable chinook salmon populations of the Snake and Klamath Rivers: limitations of negative data. Fish. Bull. **90**: 770–777.

Waples, R.S., and Aebersold, P.B. 1990. Treatment of data for duplicated gene loci in mixed-stock fishery analysis. Can. J. Fish. Aquat. Sci. **47**: 2092–2098.

Ward, R.D., Billington, N., and Hebert, P.D.N. 1989. Comparison of allozyme and mitochondrial DNA variation in populations of walleye, *Stizostedion vitreum*. Can. J. Fish. Aquat. Sci. **46**: 2074–2084.

Westneat, D.F., Noon, W.A., Reeve, H.K., and Aquadro, C.F. 1988. Improved hybridization conditions for DNA "fingerprints" probed with M13. Nucleic Acids Res. **16**: 4161.

Wilson, G.M., Thomas, W.K., and Beckenbach, A.T. 1987. Mitochondrial DNA analysis of Pacific Northwest populations of *Oncorhynchus tshawytscha*. Can. J. Fish. Aquat. Sci. **44**: 1301–1305.

Winans, G.A. 1989. Genetic variability in chinook salmon stocks from the Columbia River basin. N. Am. J. Fish. Manage. **9**: 47–52.

Withler, R.E., Beacham, T.D., Watkins, R.F., and Stevens, T.A. 1994. Identification of farm and native chinook salmon (*Oncorhynchus tshawytscha*) on the west coast of Vancouver Island, British Columbia, using the nuclear DNA probe, B2–2. Can. J. Fish. Aquat. Sci. **51**(Suppl. 1): 267–276.

Wood, C.C., McKinnell, S., Mulligan, T.J., and Fournier, D.A. 1987. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Can. J. Fish. Aquat. Sci. **44**: 866–881.

Wood, C.C., Rutherford, D.T., and McKinnell, S. 1989. Identification of sockeye salmon (*Oncorhynchus nerka*) stocks in mixed-stock fisheries in British Columbia and southeast Alaska using biological markers. Can. J. Fish. Aquat. Sci. **46**: 2108–2120.

Uptake rates of food-chain and waterborne mercury by fish: field measurements, a mechanistic model, and an assessment of uncertainties

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Abstract: We develop a model of mercury uptake by age-0 yellow perch, *Perca flavescens*, through consumption and respiration, based on a published species- and age-specific bioenergetics model and published mercury kinetics models. We use the model in conjunction with field estimates of growth, diet, temperature experience, and mercury concentration to examine the seasonal patterns in methyl mercury uptake through respiration and through the food chain, and loss rates through depuration. Seasonal variation in environmental temperature, body size, diet, and prey availability create seasonal variation in the rate of methyl mercury uptake and in the proportion of uptake from aqueous and food sources. Methyl mercury uptake was primarily from aqueous sources during the spring and fall and was dominated by food sources in the summer. Key uncertainties in our understanding of the processes of mercury uptake by fish include (*i*) quantitative relationships between water chemistry and respiratory uptake, (*ii*) quantitative estimates of intestinal tract methylation, (*iii*) empirical measures of the allometry of mercury depuration, and (*iv*) the degree of seasonal variability in mercury speciation and methylation–demethylation processes. These uncertainties hinder our ability to more fully understand and quantify the sources and pathways of uptake of mercury by fish.

Résumé : Nous sommes en train de développer, pour la perchaude de moins d'un an, *Perca flavescens*, un modèle de l'absorption du mercure par l'alimentation et la respiration, basé sur un modèle bioénergétique publié, spécifique pour l'espèce et l'âge, ainsi que sur des modèles publiés de la cinétique du mercure. Nous utilisons notre modèle conjointement à des valeurs in situ estimées de croissance, de régime alimentaire, de régime thermique et de concentration de mercure afin d'étudier les profils saisonniers de l'absorption du méthylmercure par la respiration et la chaîne alimentaire, ainsi que les taux des pertes par dépuration. Les variations saisonnières des températures du milieu, des tailles corporelles, des régimes alimentaires et de la disponibilité des proies sont à l'origine de variations saisonnières dans le taux d'absorption du méthylmercure et dans la proportion de l'absorption à partir de sources hydriques et alimentaires. L'absorption de méthylmercure provient surtout de sources hydriques au printemps et à l'automne, et principalement de sources alimentaires en été. Les principales incertitudes concernant les processus de l'absorption du mercure par les poissons sont notamment (*i*) les relations quantitatives entre la chimie de l'eau et l'absorption par la respiration, (*ii*) les estimations quantitatives de la méthylation dans les voies intestinales, (*iii*) les mesures empiriques de l'allométrie de la dépuration du mercure, et (*iv*) le degré de la variabilité saisonnière des processus de formation des espèces du mercure et des processus de méthylation–déméthylation. Ces incertitudes nous empêchent de bien comprendre et de mieux quantifier les sources et les voies d'absorption du mercure chez les poissons.
[Traduit par la Rédaction]

Introduction

During the last 10–15 years there has been substantial effort expended to understand the processes involved in the bioaccumulation of contaminants in aquatic biota. The impetus for this work has been the observation that contaminant concentrations in commercial and sport fishes frequently exceed guidelines for human consumption. Of the various contaminants pervasive in aquatic systems, mercury has received the most attention. Mercury has been found in measurable quantities in most aquatic environments, derived from a combination of natural and anthropogenic sources. Mercury in food poses a major human health risk and has been the cause of closures of sport and commercial fisheries. Many North American provinces and states have established human and animal consumption guidelines for fishes from natural waterways. Therefore, there has been an emphasis on research on the sources and processes involved in bioaccumulation of mercury in aquatic biota.

Two different approaches have been used to understand contaminant bioaccumulation processes. The first, and most frequently adopted, approach has been to survey streams, lakes, and watersheds and to correlate contaminant body

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Table 1. Location, morphometry, and water chemistry of the study lakes.

Lake	Location	Area (ha)	Depth (m)		Alkalinity ($mg \cdot L^{-1}$)	Calcium ($mg \cdot L^{-1}$)	DOC ($mg \cdot L^{-1}$)	pH
			Mean	Max.				
Harp	45°23′N, 79°08′W	66.9	12.4	40.0	3.3	3.0	4.2	6.56
Dickie	45°09′N, 79°05′W	93.2	5.0	12.0	0.7	2.5	5.1	5.82
Clear	45°12′N, 78°43′W	96.0	12.4	33.0	0.3	2.6	1.9	5.81

Note: Chemical data are whole-year arithmetic means as reported by Suns et al. (1987).

burdens in biota with geochemical, physical, and biological descriptors of aquatic systems (Suns et al. 1980, 1987; MacCrimmon et al. 1983; Wren and MacCrimmon 1986; Bodaly et al. 1987; McMurtry et al. 1989; Allard and Stokes 1989; Cope et al. 1990; Rasmussen et al. 1990; Jackson 1991; Miller et al. 1992; Lange et al. 1993). The second approach has been to examine the biological processes hypothesized to be involved in contaminant uptake by biota. Many of these process-oriented studies have been carried out in the laboratory where controlled experiments are possible (Sharpe et al. 1977; Phillips and Buhler 1978; Rodgers and Beamish 1981; Boudou and Ribeyre 1985; Cuvin and Furness 1988; Newman and Doubet 1989; Rissgard and Hansen 1990). Several studies have involved enclosure or whole-lake experiments designed to test hypotheses about uptake processes (Rudd and Turner 1983; Verta 1990; Watras and Bloom 1992). A few process-oriented studies have involved a combination of field measurements and mechanistic simulation models to quantify the influence of the various processes on contaminant uptake rates (Norstrom et al. 1976; Rodgers and Quadri 1982; Mathers and Johansen 1985; Borgmann and Whittle 1992). Process-oriented models generally include a combination of animal energetics that explicitly model respiration and (or) consumption processes and contaminant uptake kinetics. Bioenergetic rates and uptake kinetics are either determined from first principles or quantified by laboratory experimentation.

The importance of direct uptake from the water through respiration and food chain uptake through consumption of contaminated prey remains controversial (Norstrom et al. 1976; Rodgers and Quadri 1982; Spry and Wiener 1991; Borgmann and Whittle 1992). Respiratory uptake has been identified as substantial (Huckabee et al. 1979; Phillips and Buhler 1978) but these laboratory studies typically have involved aqueous concentrations that were orders of magnitude greater than those typically found in the field. Field studies of mercury uptake in piscivorous fish have identified prey consumption as the primary uptake pathway (Phillips et al. 1980; Mathers and Johansen 1985; Grieb et al. 1990; Lindquist et al. 1991). In a recent review, Spry and Wiener (1991) concluded that "the direct uptake of aqueous methyl mercury probably accounts for little — perhaps less than 10% — of the mercury accumulated in fish." In contrast, Parks et al. (1994) suggested that "mercury sources and pathways . . . are numerous, complex and poorly understood" and that "the relative importance of food and water pathways require substantial information to ascertain." We propose that Spry and Wiener's (1991) conclusion may not be generally true for mercury-contaminated fish for two reasons. First, most published field data deal with piscivorous fish that feed on prey that are highly contaminated themselves (i.e., high concentration factors of aqueous to prey mercury concentration). On the other hand, planktivorous fish feed on relatively uncontaminated prey (i.e., low concentration factors of aqueous to prey mercury concentration). Therefore, planktivorous fish would probably have a higher proportional accumulation from aqueous sources. The second reason that Spry and Wiener's (1991) general conclusion may be inappropriate involves the physiological and ecological processes of respiration and consumption, which are strongly dependent on temperature and body mass. The interaction between these processes and mercury bioaccumulation is poorly understood and typically overlooked (Borgmann and Whittle 1992), although it forms the core information required to accurately quantify the contribution of aqueous and food chain mercury uptake.

In this study we will examine the mechanisms of mercury bioaccumulation as controlled by the processes of respiration and prey consumption in nonpiscivorous, age-0 yellow perch, *Perca flavescens*. We use age-0 fish to examine these processes because, owing to their small size, their specific respiration and consumption rates are substantially higher than those of older and larger fish (Post 1990), and therefore they will accumulate mercury at higher rates, providing a more sensitive system for examining uptake processes.

We will develop a model of mercury uptake through consumption and respiration based on a published species- and age-specific bioenergetics model and mercury kinetics. The model will be applied to three populations of age-0 yellow perch that vary in growth rates and mercury contamination, and from which we collect data on diet, growth, and mercury concentration. The contribution of waterborne and consumed mercury to the mercury uptake rate and the relationship between diet and mercury uptake rate will be examined. This analysis will elucidate the magnitude of mercury uptake rates as they are affected by respiration, consumption, growth, and diet and modified by environmental temperature and bioenergetic allometry.

Methods

Field and laboratory methods

Age-0 yellow perch were captured in Harp, Dickie, and Clear lakes at monthly intervals from June through October 1985. The lakes are oligotrophic, softwater lakes located in central Ontario (Table 1). Age-0 yellow perch in open water were captured with a fine mesh (1 × 1 mm) purse seine (30 m long, 9 m deep) similar in design to that described by Evans and Johannes (1988). Once the age-0 yellow perch migrated into the littoral zone they were captured with a fine mesh (1 × 1 mm) beach seine (30 m long, 1.6 m deep). A minimum of four purse seine and four beach seine sites were sampled on each sampling date for each lake. All age-0 yellow perch captured were measured for total length to the nearest millimetre and weighed to the nearest 0.01 g wet weight. They were then frozen for subsequent diet and mercury body burden analyses.

In the laboratory, a random subsample of 10–20 fish per lake for each date were selected from the sample site with the greatest abundance of age-0 yellow perch and their stomach contents removed and pooled for a diet description. Zooplankton in the pooled diet sample were identified to genus, counted, and measured using a computer-assisted caliper system (Sprules et al. 1981; Post 1984) and biomass was calculated using genus-specific length to weight regressions (Post 1984). If the total number of zooplankters in the sample exceeded 500, a subsample of 300–500 zooplankters was analyzed as above. All benthic organisms in the pooled diet samples were identified to order and weighed to the nearest 0.0001 g wet weight. The relative proportion of zooplankton and benthos in the diet was calculated on a wet weight basis.

Temperature was measured at 1-m depth intervals at the central station of each lake and a mean epilimnetic temperature calculated for the dates when the age-0 yellow perch were abundant in the open water. Once the fish migrated to the littoral zone, temperature was measured at a depth of 0.5 m at each beach seine site and averaged amongst sites to determine a mean littoral zone temperature.

Total mercury concentration was measured for 30 individual age-0 yellow perch captured in the habitat with the highest catches per month per lake. Total mercury was determined using a hot digestion and cold vapor flameless atomic absorption spectrophotometry (Hendzel and Jamieson 1976). For small individual fish (<0.3 g) a pooled sample of approximately 0.3–0.5 g of fish was analyzed. For larger fish, a section from the caudal peduncle was analyzed. To test the hypothesis that the mercury concentration measured from the caudal peduncle sections did not differ from whole body concentrations, a subsample of 25 fish representing the complete range of fish sizes was selected for further analysis. Each of these individuals was sectioned into 2–5 sections, including the standard caudal peduncle section, and weighed and analyzed for mercury separately. A weighted whole body mercury concentration was calculated for each fish from the section concentrations. There was no significant difference between the mercury concentration measured in the caudal peduncle section and that measured for the whole body (paired t test, $t = 1.77$, $p > 0.05$). We therefore conclude that the mercury concentration in the caudal peduncle sections represents the whole body mercury concentration.

Model development

Bioenergetics

The bioenergetics model used was based on those of Kitchell et al. (1977) and Hewett and Johnson (1987) as modified by Post (1990) specifically for age-0 yellow perch. The model is an energy budget that balances energy intake through energy consumption with growth, and energy costs and losses through respiration, specific dynamic action, egestion, and excretion. Growth is modeled as $dB/dt = \text{CON} - (\text{RESP} + \text{SDA} + F + U)$, where B is fish biomass, CON is consumption, RESP is respiration, SDA is specific dynamic action, F is egestion, and U is excretion. Consumption and respiration are temperature- and biomass-dependent functions. Specific dynamic action, egestion, and excretion are modeled as constant proportions of energy consumed. The units modeled are energy on a wet weight specific basis and it is assumed that the energy contents of age-0 yellow perch and their invertebrate prey are equivalent.

The bioenergetic model coefficients used in this analysis are those presented by Kitchell et al. (1977) and Hewett and Johnson (1987) except those modified by Post (1990) for specific application to age-0 yellow perch. Respiration is modeled as $\text{RESP} = a_1 \cdot W^{b_1} \cdot \text{ACT} \cdot \text{ED} \cdot \text{OXY} \cdot \text{TDEP}_r$, where RESP is respiration in units of mg $O_2 \cdot g^{-1} \cdot d^{-1}$, a_1 and b_1 are allometric coefficients from Kitchell et al. (1977) and Post (1990), ACT is an activity multiplier determined in the field by Post (1990), ED is the energy density of the age-0 yellow perch and is assumed to be a constant 0.620 kcal·g^{-1} (1 kcal = 4.18 kJ (Treasurer 1989; Post 1990)), OXY is the oxycalorific coefficient of 0.003 24 kcal·mg O_2^{-1}, and TDEP_r is a temperature-dependent multiplier for respiration as presented in Kitchell et al. (1977) and Hewett and Johnson (1987). Consumption is modeled as $\text{CON} = a_2 \cdot W^{b_2} \cdot P \cdot \text{TDEP}_c$, where CON is consumption in units of g·g^{-1}·d^{-1}, a_2 and b_2 are allometric coefficients from Post (1990), P is the proportion of the maximal consumption rate achieved (which is fitted iteratively), and TDEP_c is a temperature-dependent multiplier for consumption as presented in Kitchell et al. (1977) and Hewett and Johnson (1987). SDA, F, and U are modeled as constant proportions of daily consumption as done by Post (1990).

Table 2. Parameter values and sources used in the bioenergetics and mercury kinetic models for examining mercury concentration in age-0 yellow perch.

Parameter	Value	Source
	Bioenergetics model	
a_1	0.035	Kitchell et al. 1977
b_1	−0.20	Kitchell et al. 1977
ACT	4.4	Post 1990
TDEP_r	See function	Kitchell et al. 1977
a_2	0.51	Post 1990
b_2	−0.42	Post 1990
TDEP_c	See function	Kitchell et al. 1977
	Mercury kinetics model	
a_3	1.445	Rodgers and Beamish 1981
b_3	1.134	Rodgers and Beamish 1981
k	0.8	See text
a_4	0.029	Sharpe et al. 1977
b_4	−0.58	Sharpe et al. 1977

Note: For definitions of parameters, see text.

This particular form of bioenergetics model has two important advantages over other approaches. First, it has been demonstrated that it accurately simulates growth and consumption dynamics in the field (Rice and Cochran 1984; Post 1990). Secondly, it explicitly models both respiration and consumption processes, which are the potential mercury uptake vectors for free-swimming fish. Therefore, when concatenated to laboratory-derived mercury uptake kinetic functions, the bioenergetics model can be used to quantitatively examine the interactions among respiration, consumption, growth, environmental temperature, and mercury body burdens of wild populations of fish.

Mercury kinetics

We develop the mercury kinetics component of the uptake model using units of methyl mercury. We assume that methyl mercury is taken up through the gills as a function of daily O_2 uptake rates and across the intestinal wall as a function of daily food consumption rates and is eliminated from the fish as a function of its wet weight.

We modeled uptake of waterborne methyl mercury using an empirical relationship derived for rainbow trout (*Oncorhynchus mykiss*) exposed to waterborne methyl mercury (Rodgers and Beamish 1981), as follows: $\log P = a_3 + b_3 \cdot \log(Q \cdot \text{Cpw})$, where P is the rate of methyl mercury uptake (ng·h^{-1}), a_3 and b_3 are regression coefficients (Table 2), Q is the rate of O_2 consumption (mg·O_2·h^{-1}), and Cpw is the methyl mercury concentration in the water (ng·mL^{-1}). Because the uptake rates of waterborne and food-derived mercury are independent (Phillips and Buhler 1978) we modeled them as additive. As the respiration rates calculated with the bioenergetics model were in units of mg·O_2·d^{-1}, we used $Q/24$ as in Rodgers and Beamish's (1981) equation and $P \cdot 24$ for calculating daily bioaccumulation rates.

Another approach that has been used to model waterborne mercury uptake involves making assumptions about the efficiency of O_2 uptake from water and the relative efficiency of mercury uptake in relation to O_2 uptake efficiency (Fagerstrom and Asell 1973; Norstrom et al.

1976; Phillips and Buhler 1978). Instead, we have adopted the empirical relationship developed by Rodgers and Beamish (1981), which has two advantages. We avoid the uncertainties involved in assumptions of transfer efficiencies and we can estimate waterborne mercury uptake directly from respiration rate estimates derived from the bioenergetics model.

Rodgers and Beamish (1981) demonstrated that environmental temperature had no direct effect on the uptake of waterborne methyl mercury, only an indirect effect, through the temperature dependence of respiration. We modeled this explicitly in the bioenergetics model. They also demonstrated that the O_2 concentration in well-aerated water had no effect on the uptake rate of waterborne methyl mercury.

Methyl mercury derived from the food web was modeled using daily food consumption rates calculated using the bioenergetics model. The assimilation of methyl mercury from ingested food was modeled as ASSIM = k·CON, where ASSIM is the quantity of methyl mercury assimilated in units of g·d^{-1}, k is the assimilation rate, and CON is consumption in units of g·g^{-1}·d^{-1}. The assimilation rate of methyl mercury from food in the intestinal tract is high, ranging from 68 to 90% (Norstrom et al. 1976; Phillips and Buhler 1978; Boudou and Ribeyre 1985; Borgmann and Whittle 1992). We therefore used an absorption efficiency of 0.8, as did Norstrom et al. (1976).

Mercury depuration probably occurs through both egestion and excretion, although there is some controversy in the literature about how to model it. Sharpe et al. (1977) demonstrated experimentally that methyl mercury depuration rates in goldfish are body size dependent over greater than two orders of magnitude in body weight. This formulation was accepted by Norstrom et al. (1976) and Borgmann and Whittle (1992). No size dependence was found in experiments with mosquito fish, *Gambusia affinis*, ranging over more than one order of magnitude in body weight (Newman and Doubet 1989). Also, in fitting observed to modeled mercury concentration, Borgmann and Whittle (1992) demonstrated that a body weight exponent of 0.0 fitted the data better, indicating no body size effect. As a first approximation of methyl mercury depuration rates we used the empirical relationship derived by Sharpe et al. (1977), which has been clearly quantified: DEP = a_4·W^{b_4}, where DEP is depuration rate in μg·d^{-1}, a_4 and b_4 are regression coefficients, and W is body wet weight in g. To explore the effect of a depuration rate that is not body size dependent, as suggested by the work of Neuman and Doubet (1989) and Borgmann and Whittle (1992), we also ran the model with $b_4 = 0$ using an a_4 that gives the same DEP value at the mean observed wet weight of age-0 yellow perch from each lake.

Model integration

The bioenergetics model was used in combination with field estimates of growth rates and temperatures to calculate daily respiration and consumption rates. Daily respiration rates were used in combination with methyl mercury uptake kinetics and an estimate of the concentration of methyl mercury in the water (see below for details) to calculate the daily uptake of methyl mercury through respiration. Daily consumption was partitioned into zooplankton and benthos (see below for details) using linear interpolations of the diet over the season, determined from field sampling in combination with an assimilation rate and an estimate of the methyl mercury concentration of mercury in zooplankton and benthos, to calculate daily uptake from food. The daily body burden of methyl mercury was calculated as $\text{BURDEN}_{i+1} = (\text{BURDEN}_i - \text{DEP}) + P_{i+1} \cdot 0.024 + \text{ASSIM}_{i+1}$, where BURDEN is the body burden in μg of methyl mercury, and DEP, P, and ASSIM are as defined earlier. P is multiplied by 0.024 to change from units of ng·h^{-1} to μg·d^{-1}. The methyl mercury concentration was calculated from mercury body burden divided by wet mass on a daily basis.

Methyl mercury concentrations in the water, zooplankton, and benthos were not available for our study lakes in the year of our study. We therefore did a series of simulations with a complete factorial design of combinations of water, zooplankton, and benthos methyl mercury concentrations, and for each combination we calculated predicted daily body burdens. For each combination of water, zooplankton, and benthos methyl mercury concentrations we compared the predicted fish methyl mercury concentration with the observed concentration and calculated the sum of squared deviations (SSDev). Note that our empirical measurements of mercury concentration in age-0 yellow perch are total mercury concentrations. Because all or most of the mercury accumulated in fish tissues is methyl mercury (Grieb et al. 1990; Sorensen 1991; Spry and Wiener 1991; Wren and Stephenson 1991; Bloom 1992; Parks et al. 1994), we assumed that the mercury measured as total mercury in age-0 yellow perch was exclusively methyl mercury. The ranges of methyl mercury concentrations that we simulated were logarithmic increments from 10^{-9} to 10^{-1} ng·mL^{-1}, from 10^{-9} to 10^{-1} g·g^{-1}, and from 10^{-9} to 10^{-1} g·g^{-1} for water, zooplankton, and benthos, respectively. First, over this broad range of concentrations, we identified the combination of water, zooplankton, and benthos mercury concentrations that gave the lowest SSDev. Then, we increased the precision of these predictions by simulating within an order of magnitude around these initial predictions using arithmetic intervals. The result of these simulations represents the combination of methyl mercury sources that give the best fit to the observed seasonal patterns in age-0 yellow perch mercury concentrations.

Field results

Age-0 yellow perch in all three study lakes grew rapidly in length in the spring and summer to an asymptote in the fall (Fig. 1). A von Bertalanffy growth curve was fitted to the length data and asymptotic lengths were 64.1, 61.3, and 57.3 mm total length and weights were 2.3, 1.9, and 1.6 g wet weight for Harp, Dickie, and Clear lakes, respectively.

The seasonal temperature profiles of the study lakes were similar, with maximal differences in temperature among lakes of 2.5°C during June, July, and August, and minimal differences in September and October (Fig. 2). Clear Lake was the slowest to warm in the spring and summer and slowest to cool in the fall.

The seasonal ontogeny of age-0 yellow perch diet was similar in the three study lakes (Fig. 3). Diet in June in all lakes and in July in Harp and Dickie lakes was exclusively zooplankton. Benthic invertebrates were added to the diet in Clear Lake in July and all lakes in August and September. In October, Harp and Dickie age-0 perch continued to eat some benthic invertebrates but in Clear Lake the diets consisted exclusively of zooplankton.

Model simulations

We calculated the daily growth rates of age-0 yellow perch in Clear, Dickie, and Harp lakes from the slope of the von Bertalanffy growth curves (Fig. 4). Daily growth rates in the three lakes were similar in the 1st month but diverged in the later 3 months. Age-0 yellow perch in Harp Lake had the highest growth rates and those in Clear Lake had the lowest growth rates during this period.

Daily estimates of ambient temperature from linear interpolations (Fig. 2) and daily growth rates (Fig. 4) were combined in the bioenergetics model to calculate daily respiration and consumption rates. The seasonal patterns of respiration and consumption (Fig. 4) in each of the lakes were similar. In general, respiration and consumption rates increased through the first half of the growing season as the fish grew and as

Fig. 1. Growth in length of age-0 yellow perch in Harp, Dickie, and Clear lakes. Measured mean length ±1 standard deviation and a fitted von Bertalanffy growth curve are indicated. The coefficients of the von Bertalanffy growth curve are indicated.

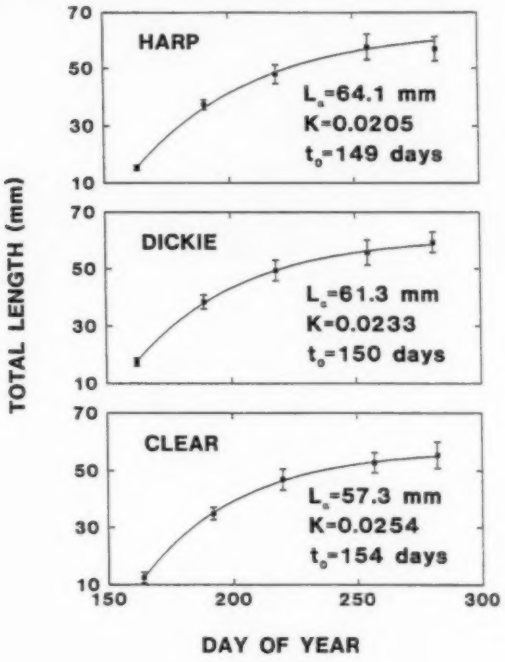


Fig. 2. Temperature experienced by age-0 yellow perch in Harp, Dickie, and Clear lakes. The first data point for all lakes is the mean epilimnetic temperature at the lake center and subsequent points are mean littoral zone temperatures at 0.5 m depth (at 3–5 beach seine sites).

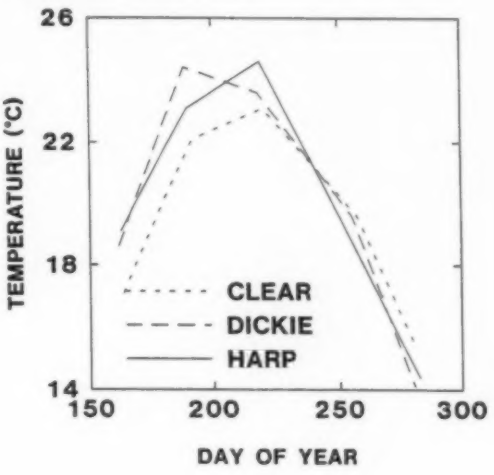
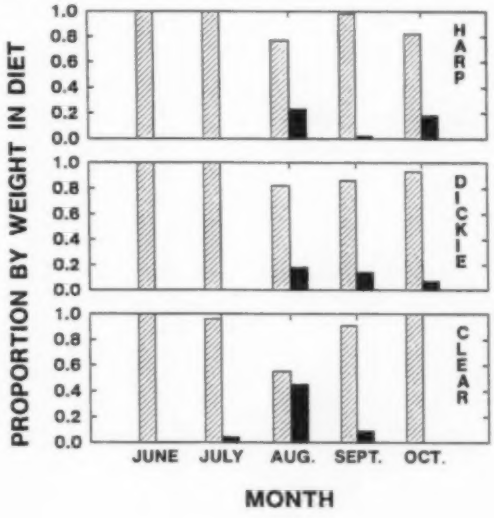


Fig. 3. Proportion by weight in the diet of age-0 yellow perch in Harp, Dickie, and Clear lakes of zooplankton (hatched bars) and benthos (solid bars).



temperatures rose, and decreased in the second half of the growing season as temperatures declined. Rates did not decline as markedly in the fall in Harp Lake because the fish were growing at higher rates and had attained a larger mass than fish in the other lakes.

The integrated bioenergetics and mercury kinetics model was used to predict methyl mercury concentration in age-0 yellow perch using combinations of methyl mercury concentrations in the water, zooplankton, and benthos. The SSDev between observed and predicted values was calculated and plotted as a function of the mercury concentration in the water and the mercury concentration of the food (a weighted average food concentration, given the proportional diet and mercury concentration in zooplankton and benthos) (Fig. 5). The minimum SSDev, and therefore the best fit to the observed seasonal pattern in whole body methyl mercury concentration in age-0 yellow perch, was in a trough of minima parallel to the food mercury concentration axis for both Harp and Dickie lakes. This indicates that the seasonal methyl mercury concentration trajectory is more similar to the seasonal pattern in uptake through consumption (which is a combination of seasonal consumption rates, seasonal diet, and methyl mercury concentration in the food items) than it is to the seasonal pattern in respiration uptake (which is a combination of the seasonal pattern in respiration and methyl mercury concentration in the water) in Harp and Dickie lakes. Clear Lake shows the opposite pattern. The minimum SSDev is in a trough of minima parallel to the water methyl mercury concentration axis. This indicates that the seasonal body burden trajectory is more similar to the seasonal pattern in respiration than it is to the seasonal pattern in consumption uptake in Clear Lake. The pattern of the sum of squared deviations in all three lakes shows secondary troughs (with intermediate SSDev) perpendicular to the main troughs. This suggests that a single uptake vector, be it food or water, is not adequate to fully explain methyl mercury uptake rates, but rather both vectors contribute, but in different ratios throughout time within lakes and among lakes.

Fig. 4. Daily growth rates (calculated as the slope of the von Bertalanffy growth curve), daily respiration rates (calculated using the bioenergetics model with daily growth rates and temperature experience), and daily consumption rates (calculated using the bioenergetics model with daily growth rates and temperature experience) of age-0 yellow perch in Harp, Dickie, and Clear lakes.

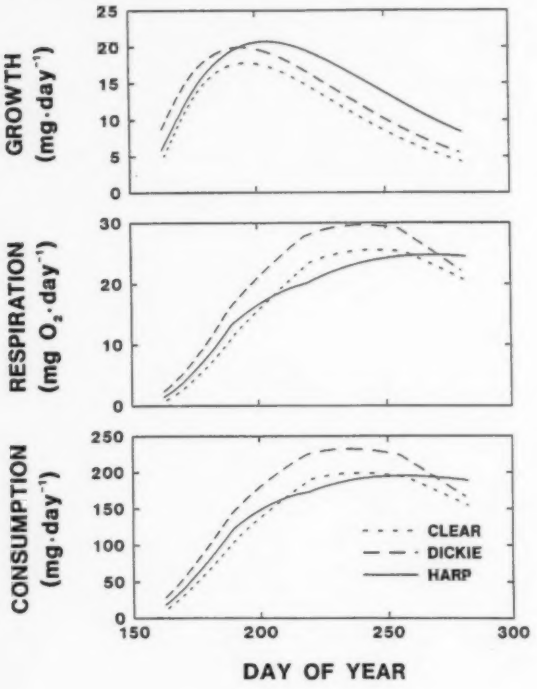
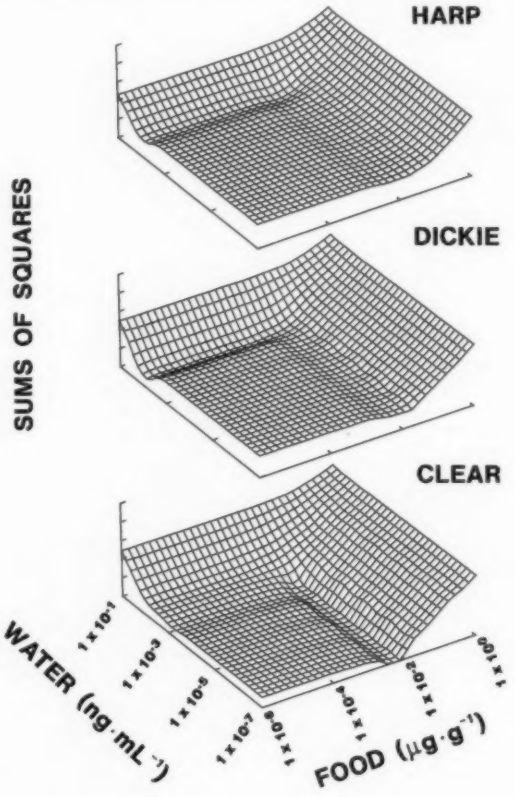


Fig. 5. Contour plots of log sums of squared deviations (SSDev) between predicted and observed seasonal mercury body burdens at seven logarithmic intervals of water mercury concentration and food mercury concentrations for Harp, Dickie, and Clear lakes. The mercury concentration of the food is the average of the concentrations in zooplankton and benthos weighted by the proportional diet composition.



The methyl mercury concentrations in water, zooplankton, and benthos that produced the best fits (minimal SSDev) are presented in Table 3. Harp Lake had the highest concentrations in all three pools, with Dickie Lake concentrations somewhat lower, but in the same order of magnitude. Despite the differences in mercury concentrations among lakes, the concentration factors (biota concentration divided by water concentration) were similar among lakes, lending credence to the simulation results (Fig. 6). The fit was approximately 100 times better in Clear Lake (see SSDev in Table 3) than in the other two lakes but the relative sizes of the predicted methyl mercury pools made little sense. In Clear Lake, the best fit was obtained with a zooplankton concentration 10 times higher and water and benthos concentrations 100 times lower than in Harp and Dickie lakes. This result is unlikely, because the mercury concentration in benthos is typically higher than in zooplankton in the same water body. We therefore did a second simulation for Clear Lake in which we assumed that (*i*) the relationship between water mercury concentration and final fall body burden in age-0 yellow perch is the same in Clear Lake as in Harp and Dickie lakes and (*ii*) the ratios of zooplankton to water and benthos to water are the same in Clear Lake as in Harp and Dickie lakes (Table 3). With these assumptions, the quality of the Clear Lake fit was reduced somewhat but was still better than that for Harp and Dickie lakes. It is probably inappropriate to overinterpret the results of the Clear Lake simulations. The observed body burden in Clear Lake was low and showed little seasonal variance. Therefore, the Clear Lake example provides a much less sensitive test of the model that is driven by the seasonal dynamics of respiration, consumption, and diets than do the Harp and Dickie data sets. The seasonal patterns in observed and predicted mercury concentration of age-0 yellow perch were similar (Fig. 7) and we conclude that the model is capable of giving reasonable fits to field data.

Improved agreement between observed and predicted mercury body burden may have been attained if we collected diet data more frequently. The rapid increase in mercury concentration between August and September in Harp and Dickie lakes is correlated with the addition of benthos to the diet in August. We assumed a linear relationship between diet composition and time and may have therefore overestimated mercury accumulation from benthos between July and August if there was a more discrete shift to benthos. If this hypothesis is

Table 3. Predicted concentration of methyl mercury in water, zooplankton, and benthos that minimized the sum of squared deviations (SSDev) between predicted and observed seasonal trajectories in mercury concentration of age-0 yellow perch in Harp, Dickie, and Clear lakes.

Lake	Water ($\mu g \cdot g^{-1}$)	Zooplankton ($\mu g \cdot g^{-1}$)	Benthos ($\mu g \cdot g^{-1}$)	SSDev
Harp	10	0.003	0.11	0.205
Dickie	5	0.002	0.10	0.0616
Clear				
Simulation 1	0.07	0.010	0.003	0.0023
Simulation 2	1.4	0.0005	0.021	0.012

Note: The Harp, Dickie, and Clear lake (simulation 1) values are for the lowest SSDev. Clear Lake simulation 2 assumes that (*i*) the relationship between water mercury concentration and final fall mercury concentration is the same in Clear Lake as in Harp and Dickie lakes and (*ii*) the concentration factors for zooplankton and benthos are the same in Clear Lake as in Harp and Dickie lakes.

correct, agreement between observed and predicted would be better in August. An increase in the benthos mercury concentration that gave the best fit would then reduce the difference between observed and predicted concentrations in September and October. These observations suggest that if there are diet shifts between prey organisms that vary in mercury concentration, it is necessary to characterize the magnitude of the diet shift with a resolution finer than that provided by monthly sampling.

The seasonal patterns in the proportion of the mercury uptake through respiration and consumption reflect seasonal patterns in temperature, body size dependent energetics, and diet (Fig. 8). When growth rates (Fig. 4) and temperatures (Fig. 2) were low, in the spring and fall, the largest proportion of mercury uptake was through respiration. In the summer, when temperature and consumption rates were the highest, the proportion of mercury uptake from consumption exceeded that through respiration. The partial switch in diet from zooplankton to benthos in July or August was largely responsible for the summer peaks in the proportion of mercury uptake from consumption. In Clear Lake, in August, when approximately 45% of the diet was benthos, mercury uptake through consumption was about 80% of total uptake. Therefore, there is dramatic seasonal variation in the proportional uptake of mercury by respiration and through the food chain. This seasonal pattern is driven by three processes: (*i*) the temperature dependence of respiration and consumption, (*ii*) the allometry of respiration and consumption, and (*iii*) the seasonal abundance of prey. In aggregate, these processes create a seasonally variable ratio of uptake from water and food. The temperature dependence of the proportional mercury uptake through respiration and the food chain can be understood by examining the temperature dependence of each process. The Q_{10} for consumption is higher than for respiration, which demonstrates that respiratory mercury uptake would dominate at low temperatures and consumption would dominate at temperatures near the optimum for growth. The allometric component of the seasonal pattern is more difficult to discern from our data because not only is mass changing more than 100-fold from spring to fall, but water temperature and prey availability are also changing over the same time period. Theoretically, scope

Fig. 6. Concentration factors for zooplankton/water, benthos/water, and age-0 yellow perch/water for Harp, Dickie, and Clear (simulation 2) lakes.

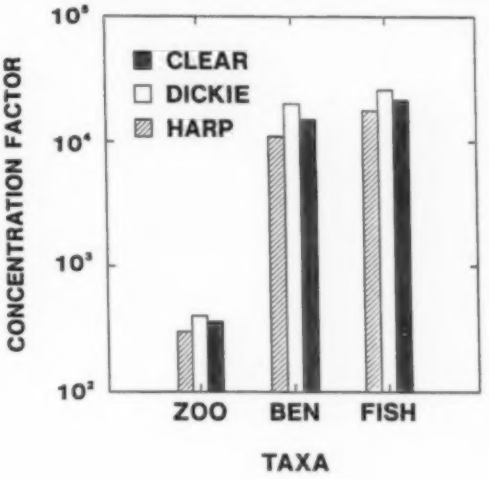
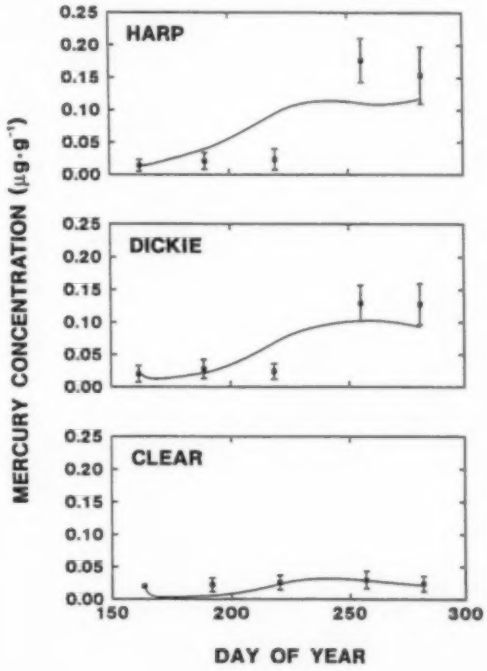
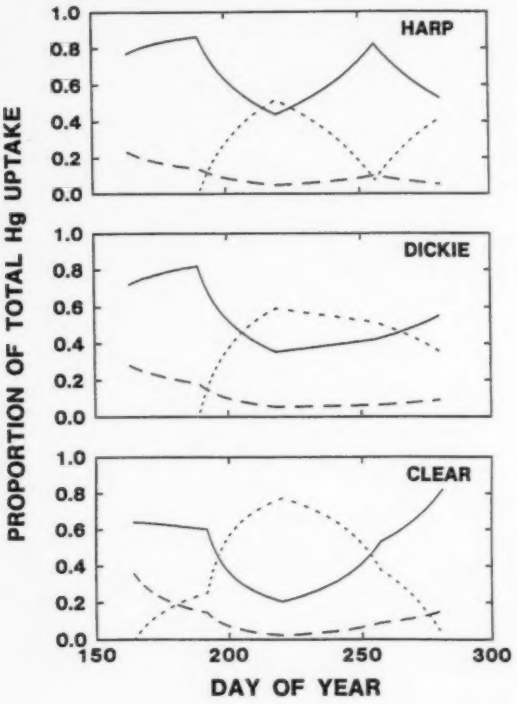


Fig. 7. Observed (mean ±1 standard deviation) and predicted (solid line) mercury concentration of age-0 yellow perch in Harp, Dickie, and Clear (simulation 2) lakes.



for growth (the energy content of consumed food that is assimilated minus the energetic costs as measured by respiration) declines with mass (Post 1990), suggesting that uptake of mercury from the food chain should dominate for small fish and decline with increases in mass. The combination of low

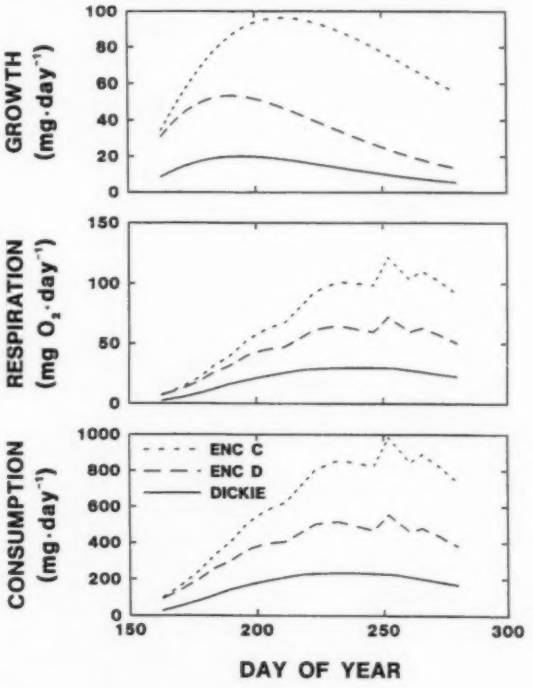
Fig. 8. Proportion of total daily uptake of mercury from the water (solid line), zooplankton (dashed line), and benthos (dotted line) by age-0 yellow perch in Harp, Dickie, and Clear (simulation 2) lakes.



spring water temperatures (Fig. 2) and low growth and consumption rates creates a dominance of uptake through respiration for the smallest age-0 yellow perch in the spring.

The contrast in age-0 yellow perch growth among Harp, Dickie, and Clear lakes is not large when compared with results from a broader set of lakes (Post and McQueen 1994). The maximal body mass that we observed in the fall was 2.3 g in Harp Lake but age-0 yellow perch have been observed in the fall to exceed 10 g (Post and McQueen 1994). To understand the influence of growth rate in general (and therefore consumption rates) on mercury bioaccumulation we now contrast mercury uptake rates and proportional respiratory and food chain uptake rates between our study lakes and other systems that support faster growth rates of age-0 yellow perch. Post and McQueen (1994) provide temperature profiles and growth trajectories for age-0 yellow perch with fall asymptotic masses of 4.3 and 9.7 g that were grown in large enclosures at various densities. We used their in situ enclosure growth, consumption, and respiration rate data in concert with our estimated in situ mercury concentrations in water, zooplankton, and benthos and seasonal diet data from Dickie Lake to calculate the mercury concentration and the proportional uptake from food for these faster growing populations. As observed in our study lakes, the enclosure-grown yellow perch had seasonally varying growth, respiration, and consumption rates (Fig. 9). In our study lakes, as characterized by Dickie Lake, yellow perch had relatively low growth, respiration, and consumption rates, enclosure D (ENC D, Fig. 9) had intermediate rates, and enclosure C (ENC C, Fig. 9) had high rates. This range in growth rates (an increase of 150-to 1000-fold in mass from spring to fall) represents the range observed across the geographic range of yellow perch (Post and McQueen 1994). Despite substantial differences in the growth, respiration, and consumption rates of age-0 yellow perch grown in Dickie Lake and the two experimental enclosures, the differences in mercury concentration and the proportional uptake of mercury from the food chain were small (Fig. 10). There are of course substantial differences in body burden, but these simulations suggest that neither mercury concentration nor the proportional uptake of waterborne and food chain mercury varies with rates of growth and consumption. Therefore, mercury is neither diluted nor concentrated by growth but reaches an equilibrium with the mercury concentration in the environment in which the fish consume and respire. This conclusion is robust over a 5-fold range in growth rate or mass at age that is rarely exceeded in natural fish populations.

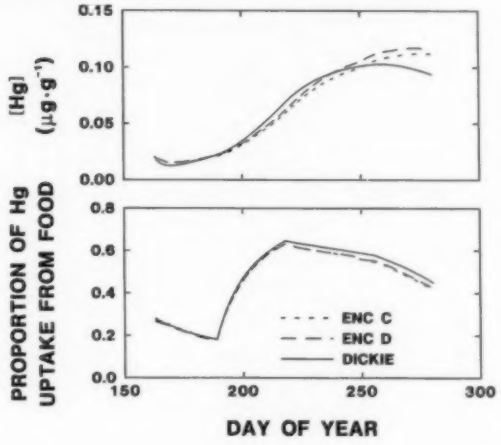
Fig. 9. Growth, respiration, and consumption rates of relatively slow growing age-0 yellow perch in Dickie Lake and faster growing age-0 yellow perch in two in situ enclosures (enclosure C, ENC C; and enclosure D, ENC D) in Lake St. George (adapted from Post and McQueen 1994). Note that the apparent higher variability in respiration and consumption rates for fish grown in enclosures is caused by more frequent temperature data collection in the enclosures than in Dickie Lake.



Uncertainties in mercury kinetics

We examined the sensitivity of the parameters in the mercury

Fig. 10. Predicted mercury concentration in age-0 yellow perch and the proportion of mercury accumulated from food in Dickie Lake and for faster growing fish in the two experimental enclosures.



kinetics component of the mercury uptake model using two response variables: (*i*) the predicted mercury concentration in the fall and (*ii*) the sum of the squared deviations for the seasonal trajectories of predicted from observed mercury concentration. The results were qualitatively similar for the two response variables. Here we present results for only the first response variable. In the Harp and Dickie Lake simulations, predicted mercury concentrations were sensitive to both the intercept (a_3) and slope (b_3) of the respiration uptake function derived in laboratory experiments by Rodgers and Beamish (1981) (Fig. 11). Predicted mercury concentrations were insensitive to the assimilation of mercury from food (k) and to the intercept (a_4) and slope (b_4) of the weight dependence of depuration in simulations for all three lakes. Results were the same for either small (5%) or large (20%) perturbations in the value of the parameters, suggesting that the model does not contain strong discontinuities. The Clear Lake simulations were insensitive to all of the mercury kinetics parameters at both levels of perturbation (Fig. 11). This reinforces a point made earlier that because the mercury concentration in Clear Lake age-0 yellow perch is low and has only small seasonal variability, it does not provide a sensitive system for examining the processes of mercury accumulation.

Controversy exists as to whether mercury depuration rate is body size dependent. We fitted both body size dependent and body size independent models to our observed mercury concentration trajectories. In the Harp and Dickie lake simulations, the body size dependent depuration model produced mercury concentrations in the fall that were closer to those observed, and the model fitted seasonal trajectories better (Table 4). Examination of the seasonal mercury concentration trajectories for Harp and Dickie lakes (Fig. 7) explains this result. In both lakes, the model overestimates mercury concentration in the first half of the season and underestimates mercury concentration later in the season. Elimination of the negative exponential body size dependent term exacerbates this seasonal pattern by decreasing depuration in the first half of the season (and therefore increasing mercury concentration)

Fig. 11. Sensitivity of the predicted mercury concentration to uncertainty in the mercury kinetics parameters. The absolute value of the percent deviation between the mercury body burden in the nominal run and the value for perturbations of ±5 and ±20% for Harp, Dickie, and Clear lakes. Values more extreme than the horizontal broken lines indicate a parameter to which model predictions are sensitive. a_3 and b_3 are the intercept and slope of Rodgers and Beamish's (1981) equation relating methyl mercury uptake from water to respiration rate and methyl mercury concentration in the water. k is the assimilation rate of methyl mercury from the food. a_4 and b_4 are the intercept and slope of the depuration allometry relationship of Sharpe et al. (1977).

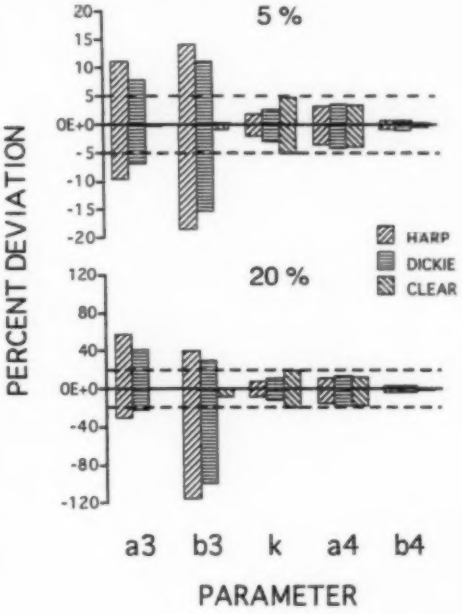


Table 4. The effect of the form of the methyl mercury depuration model (b_4 value) on the final mercury concentration in the fall and the sums of the squared deviations (SSDev) between predicted and observed mercury concentrations for Harp, Dickie, and Clear lakes.

	Harp		Dickie		Clear	
b_4	[Hg] ($\mu g \cdot g^{-1}$)	SSDev	[Hg] ($\mu g \cdot g^{-1}$)	SSDev	[Hg] ($\mu g \cdot g^{-1}$)	SSDev
–0.58	0.1172	0.2055	0.0933	0.0616	0.0323	0.0023
0	0.0970	0.3035	0.0767	0.0934	0.0267	0.0004

when the age-0 yellow perch are small, and increasing depuration rates in the second half of the season when the fish are large (and therefore decreasing mercury concentration). In the Clear Lake simulations, the body size independent depuration model produced fall mercury concentrations closer to those observed and the model fitted the seasonal trajectories better (Table 4). This improved fit with the size-independent depuration model is due to the low seasonal variability in mercury

concentration in Clear Lake as the age-0 yellow perch grow over approximately three orders of magnitude in wet weight.

We modeled the uptake of aqueous methyl mercury using an empirical relationship developed by Rodgers and Beamish (1981) who experimented with methyl mercury concentrations 10^3–10^4 times higher than commonly found in natural water. Extrapolations from their relationships may therefore involve inaccuracies. The likely bias introduced by these extrapolations would be an underestimate of transfer efficiencies and therefore an overestimate of the water concentration necessary to balance the energy and mercury budgets. In addition, Rodgers and Beamish's (1981) relationship was derived from experiments done in high alkalinity water. They demonstrated subsequently that methyl mercury uptake rates were five to six times higher in lower alkalinity water (Rodgers and Beamish 1983). More recent work has demonstrated that gill permeability and therefore uptake rates are inversely proportional to pH and calcium ion concentration (Winfrey and Rudd 1990; Boudou et al. 1991; Ponce and Bloom 1991). In addition, the presence of inorganic mercury further increased the efficiency of methyl mercury uptake (Rodgers and Beamish 1983). Because our study lakes have low alkalinity and both methyl and inorganic mercury in the water column, we would expect our predicted uptake efficiencies to be biased low, and therefore predicted water column methyl mercury concentrations to be biased high. Our calculated estimates of water column methyl mercury range across study lakes from 1.4 to 10 $ng \cdot L^{-1}$. These estimates are most likely overestimates by at least five to six times given the biases introduced by using Rodgers and Beamish's (1981) aqueous methyl mercury uptake relationship. Mierle and Ingram (1991) measured total mercury in streams entering Harp and Dickie lakes ranging from 1 to 29 $ng \cdot L^{-1}$ seasonally and suggested that dry fall to the lake is another substantial source of mercury. Recent experimental and review papers have presented reliable estimates of total and methyl mercury in natural waters. Total mercury ranges from <1.0 to 28 $ng \cdot L^{-1}$ and methyl mercury from 0.04 to 3.96 $ng \cdot L^{-1}$, and the proportion of methyl to total mercury varies from approximately 1 to 30% (Richman et al. 1988; Mierle 1990; Wiener et al. 1990; Gilmour and Henry 1991; Spry and Wiener 1991; Watras and Bloom 1992; Harris and Snodgrass 1993; Jackson 1993). Unfortunately, a mass balance of total or methyl mercury or in situ methylation rates is not available for our study lakes. Clearly, future research will have to focus on in situ rates of methyl mercury uptake as a function of ambient water chemistry, water column mercury speciation, and in situ methylation rates in an effort to understand the processes that regulate the in situ uptake of aqueous mercury. The sensitivity analysis of our mercury kinetics model clearly demonstrates the importance of having accurate estimates of the coefficients of the respiratory uptake of methyl mercury by fish.

Assessments of rates of mercury uptake through the food chain depend on a clear understanding of several processes, and on mercury availability in prey organisms. Our predictions of methyl mercury concentrations in zooplankton and benthos are contingent on the accuracy of the transfer rates we assumed in the model. We assumed that 80% of the methyl mercury in zooplankton and benthos would be absorbed by the fish intestinal tract. This assumption is well supported empirically (Phillips and Buhler 1978; Boudou and Ribeyre 1985; Boudou et al. 1991; Sorensen 1991; Borgmann and Whittle 1992). There is evidence that fish intestinal contents are involved in the methylation of inorganic mercury (Rudd et al. 1980; Ludwicki 1990). This suggests that our estimates of methyl mercury concentration in food are biased high and the estimates are likely to represent a value intermediate between methyl mercury and total mercury, depending on the efficiency of in situ intestinal tract methylation. Assuming that approximately 60% of the total mercury in invertebrates is methyl mercury (Wren and Stephenson 1991), our estimated methyl mercury concentrations in zooplankton and benthos are similar to those found in other natural lakes (Ramsey and Ramlal 1987*a*, 1987*b*; Jackson 1988; Allard and Stokes 1989; Wren and Stephenson 1991; Watras and Bloom 1992). Our estimates also corroborate the observation that bottom-dwelling invertebrates have higher mercury concentrations than water-column invertebrates (Wren and Stephenson 1991). We have no empirical data on the mercury concentration in invertebrates in our study lakes but we do have data for Mouse and Ranger lakes within 50 km of our study lakes (D.J. McQueen, unpublished data). Mercury concentrations predicted in invertebrates in Harp and Dickie lakes are 5 to 10 times higher than those observed in Mouse and Ranger lakes, suggesting that Harp and Dickie lakes are more contaminated and (or) that methylation in intestinal tracts may be important. Age-1 yellow perch in Mouse and Ranger lakes have approximately 30–50% of the total mercury concentration observed in age-0 yellow perch in the fall in Harp and Dickie lakes (D.J. McQueen, unpublished data). These uncertainties, intestinal tract assimilation of methyl mercury, in situ intestinal tract methylation rates, and mercury concentration and speciation in invertebrates must be addressed if we are to understand in more detail the process of food chain transfer of mercury.

Most field studies that have examined mercury bioaccumulation by fish have worked on an annual time scale (see reviews in Wiener et al. 1990; Gilmour and Henry 1991; Parks et al. 1991; Sorensen 1991; Spry and Wiener 1991; Wren and Stephenson 1991; Bloom 1992; Borgmann and Whittle 1992; Haines et al. 1992; Parks et al. 1994). This approach implicitly assumes that the combination of mercury bioavailability and physiological uptake processes does not vary seasonally. In our analysis we explicitly modeled the seasonally varying physiological processes inherent in mercury uptake by fish but kept the pool of available mercury invariant through the season. In doing this, we propose that the dominant processes in mercury bioaccumulation are the physiological processes that vary dramatically by season as a function of allometry and temperature dependence. It is also likely that there is seasonal variability in mercury availability. For example, there is substantial seasonal variability in the import and export of total mercury through stream input, dry fall, and stream export in Harp and Dickie lakes (Mierle 1990; Mierle and Ingram 1991). There is also seasonal variation in the in situ methylation and demethylation rates typically related to seasonal variation in algal biomass, dissolved organic carbon concentration, and microbial activity, all of which typically peak in the summer during the highest ambient temperatures (Rudd et al. 1983; Korthals and Winfrey 1987; Winfrey and Rudd 1990; Gilmour and Henry 1991; Jackson 1991, 1993). Unfortunately, there is not yet any published research that accomplishes a mass balance of mercury assessing import, export, and in situ

methylation–demethylation processes. This is basic information that is necessary to couple the physiological uptake processes to variation in the environmental availability of mercury in an effort to understand the in situ uptake kinetics with greater precision.

Conclusions

The uptake of environmental mercury by fish is a function of two processes. The rate of uptake of waterborne mercury through respiration varies with the concentration and speciation of mercury in the water, and temperature and fish body size as they affect respiratory rates. The uptake of mercury from food varies with the concentration and speciation in organisms in the diet as well as with temperature and body size as they affect rates of consumption. Because temperature, body size, and often diet vary substantially with season, the rate of mercury uptake and the relative contribution of the two uptake vectors are seasonally dynamic. In this study, during August and September, when the age-0 yellow perch partially switched their diet from zooplankton to benthos and when temperature and consumption were highest, the uptake of mercury through consumption exceeded the uptake through respiration. During the spring and fall, when diet was primarily zooplankton, with a lower mercury concentration than benthos, and lower water temperatures, the majority of mercury uptake was through respiration of waterborne mercury. In this study, we have explicitly modeled the processes involved in mercury uptake and can identify the variables likely to play important roles in uptake. Clearly, mercury concentration and speciation in the water and food are important, but if we can assume that there is an equilibrium established between mercury concentrations in water and in food organisms (because of their own uptake dynamics) then the proportional uptake rates are not affected by overall environmental mercury levels, but rather by the diet selections made by the fish. Seasonal patterns are then driven by the combination of seasonally varying diet and growth efficiency, which is highest at the optimal temperature for growth, and declines at higher or lower temperatures. At suboptimal temperatures, respiration uptake increases relative to consumption uptake because respiration rates are a larger component of the energy budget than at optimal temperatures.

Our estimates of the proportional uptake of mercury uptake from aqueous sources and through the food chain are at odds with some recent reviews and modeling studies (Gilmour and Henry 1991; Spry and Wiener 1991; Borgmann and Whittle 1992; Harris and Snodgrass 1993). We propose two reasons for this lack of agreement. First, the majority of studies that examine mercury uptake by fish focus on large-bodied piscivorous fish. This is significant because large-bodied fish have low weight-specific respiration rates and use prey that themselves have high mercury concentrations. Many species of fish, and also juveniles of piscivorous fish, are small bodied with high weight-specific respiration rates and feed on invertebrates with substantially lower mercury body burdens than fish. The second reason is that studies often ignore the fact that fish energetics are strongly variable in a seasonally varying environment. For example, Harris and Snodgrass (1993) stated that the bioenergetic coefficients that they borrowed from Norstrom et al. (1976) "were assumed to be reasonable." Unfortunately, they modeled respiration at a constant optimal temperature and assumed that consumption had the same allometry as respiration. This is equivalent to our taking a midsummer snapshot of mercury uptake for which we would also conclude that uptake of mercury from the food chain was dominant. Clearly, contaminant uptake processes, if controlled by physiological processes, are complex and dynamic and require substantial information to determine with precision (Parks et al. 1994).

There are a number of uncertainties that hinder our ability to identify sources and pathways and to model accurately the processes of mercury uptake by fish. It is clear that water chemistry affects gill permeability and therefore the uptake efficiency of aqueous mercury, but there are no quantitative relationships available to apply to the majority of aquatic systems. Experimental studies have quantified with reasonable precision the uptake of methyl mercury in the intestinal tracts of fish but the extent of in situ mercury uptake is not clear, given that prey may contain both methyl and inorganic mercury. In addition, while this study clarifies the significance of seasonal variation in the physiological processes of contaminant uptake, the magnitude of the seasonal variation in methylation processes, mercury speciation, and therefore the bioavailability of mercury to aquatic biota are not clear. Finally, the emphasis on mercury contamination by large-bodied piscivorous fish has hindered our ability to understand the links between elemental sources of mercury and the complex direct and indirect food web processes.

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References

Allard, M., and Stokes, P.M. 1989. Mercury in crayfish species from thirteen Ontario lakes in relation to water chemistry and smallmouth bass (*Micropterus dolomieui*) mercury. Can. J. Fish. Aquat. Sci. **46**: 1040–1046.

Bloom, N.S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquat. Sci. **49**: 1010–1017.

Bodaly, R.A., Strange, N.E., Hecky, R.E., Fudge, R.J.P., and Anema, C. 1987. Mercury content of soil, lake sediment, net plankton, vegetation, and forage fish in the area of the Churchill River diversion, Manitoba. Can. Data Rep. Fish. Aquat. Sci. No. 610.

Borgmann, U., and Whittle, D.M. 1992. Bioenergetics and PCB, DDE, and mercury dynamics in Lake Ontario lake trout (*Salvelinus namaycush*): a model based on surveillance data. Can. J. Fish. Aquat. Sci. **49**: 1086–1096.

Boudou, A., and Ribeyre, F. 1985. Experimental study of trophic contamination of *Salmo gairdneri* by two mercury compounds, $HgCl_2$ and CH_3HgCl, analysis at the organism and organ levels. Water Air Soil Pollut. **26**: 137–148.

Boudou, A., Delnomdedieu, M., Georgescauld, D., Ribeyre, F., and Saouter, E. 1991. Fundamental roles of biological barriers in mercury accumulation and transfer in freshwater ecosystems (analysis at organism, organ, cell and molecular levels). Water Air Soil Pollut. **56**: 807–822.

Cope, W.G., Wiener, J.G., and Rada, R.G. 1990. Mercury accumulation in yellow perch in Wisconsin seepage lakes: relation to lake characteristics. Environ. Toxicol. Chem. **9**: 931–940.

Cuvin, M.L.A., and Furness, R.W. 1988. Uptake and elimination of inorganic mercury and selenium by minnows *Phoxinus phoxinus*. Aquat. Toxicol. **13**: 205–216.

Evans, D.O., and Johannes, P.R. 1988. A bridle-less trawl and fine-mesh purse seine for sampling pelagic coregonine larvae with observations of spatial distribution and abundance. *In* Ontario fisheries technical report series No. 27. Ontario Ministry of Natural Resources, Toronto, Ont.

Fagerstrom, T., and Asell, B. 1973. Methyl mercury accumulation in an aquatic food chain: a model and some implications for research planning. Ambio, **2**: 164–171.

Gilmour, C.C., and Henry, E.A. 1991. Mercury methylation in aquatic systems affected by acid deposition. Environ. Pollut. **71**: 131–169.

Grieb, T.M., Driscoll, C.T., Gloss, S.P., Schofield, C.L., Bowie, G.L., and Porcella, D.B. 1990. Factors affecting mercury accumulation in fish in the upper Michigan peninsula. Environ. Toxicol. Chem. **9**: 919–930.

Haines, T.A., Komov, V., and Jagoe, C.H. 1992. Lake acidity and mercury content of fish in Darwin national reserve, Russia. Environ. Pollut. **78**: 107–112.

Harris, R.C., and Snodgrass, W.J. 1993. Bioenergetic simulations of mercury uptake and retention in walleye (*Stizostedion vitreum*) and yellow perch (*Perca flavescens*). Water Pollut. Res. J. Can. **28**: 217–236.

Hendzel, M.R., and Jamieson, D.M. 1976. Determination of mercury in fish. Anal. Chem. **48**: 926–928.

Hewett, S.W., and Johnson, B.L. 1987. A generalized bioenergetics model of fish growth for microcomputers. Tech. Rep. Univ. Wis. Sea Grant Coll. Program No. WIS-SG-87-245.

Huckabee, J.W., Elwood, J.W., and Hildebrand, S.G. 1979. Accumulation of mercury in freshwater biota. *In* The biogeochemistry of mercury in the environment. *Edited by* J.O. Nriagu. Elsevier – North Holland Biomedical Press, Amsterdam, The Netherlands. pp. 277–302.

Jackson, T.A. 1988. Accumulation of mercury by planktonic and benthic invertebrates in riverine lakes of northern Manitoba (Canada): importance of regionally and seasonally varying environmental factors. Can. J. Fish. Aquat. Sci. **45**: 1744–1757.

Jackson, T.A. 1991. Biological and environmental control of mercury accumulation by fish in lakes and reservoirs of northern Manitoba, Canada. Can. J. Fish. Aquat. Sci. **48**: 2449–2470.

Jackson, T.A. 1993. Effects of environmental factors and primary production on the distribution and methylation of mercury in a chain of highly eutrophic riverine lakes. Water Pollut. Res. J. Can. **28**: 177–216.

Kitchell, J.F., Stewart, D.J., and Weininger, D. 1977. Application of a bioenergetic model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. **34**: 1922–1935.

Korthals, E.T., and Winfrey, M.R. 1987. Seasonal and spatial variations in mercury methylation and demethylation in an oligotrophic lake. Appl. Environ. Microbiol. **53**: 2397–2404.

Lange, T.R., Royals, H.E., and Connor, L.L. 1993. Influence of water chemistry on mercury concentration in largemouth bass from Florida lakes. Trans. Am. Fish. Soc. **122**: 74–84.

Lindqvist, O., Johnasson, K., Aastrup, M., Anderson, A., Bringmack, L., Hovsenius, G., Håkanson, L., Iverfeldt, A., Miele, M., and Timm, B. 1991. Mercury in the Swedish environment; recent research on causes, consequences, and corrective measures. Chap. 14. Water Air Soil Pollut. **55**: 193–216.

Ludwicki, J.K. 1990. Invitro methylation and demethylation of mercury compounds by the intestinal contents. Bull. Environ. Contam. Toxicol. **44**: 357–362.

MacCrimmon, H.R., Wren, C.D., and Gots, B.L. 1983. Mercury uptake by lake trout, *Salvelinus namaycush*, relative to age, growth, and diet in Tadenac Lake with comparative data from other Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. **40**: 114–120.

Mathers, R.A., and Johansen, P.H. 1985. The effects of feeding ecology on mercury accumulation in walleye (*Stizostedion vitreum*) and pike (*Esox lucius*) in Lake Simcoe. Can. J. Zool. **63**: 2006–2012.

McMurtry, M.J., Wales, D.L., Scheider, W.A., Beggs, G.L., and Dimond, P.E. 1989. Relationship of mercury concentrations in lake trout (*Salvelinus namaycush*) and smallmouth bass (*Micropterus dolomieui*) to the physical and chemical characteristics of Ontario lakes. Can. J. Fish. Aquat. Sci. **46**: 426– 434.

Mierle, G. 1990. Aqueous inputs of mercury to Precambrian shield lakes in Ontario. Environ. Toxicol. Chem. **9**: 843–851.

Mierle, G., and Ingram, R. 1991. The role of humic substances in the mobilization of mercury from watersheds. Water Air Soil Pollut. **56**: 349–357.

Miller, P.A., Munkittrick, K.R., and Dixon, D.G. 1992. Relationship between concentrations of copper and zinc in water, sediment, benthic invertebrates, and tissues of white sucker (*Catastomus commersoni*) at metal-contaminated sites. Can. J. Fish. Aquat. Sci. **49**: 978–984.

Newman, M.C., and Doubet, D.K. 1989. Size-dependence of mercury (II) accumulation kinetics in the mosquitofish, *Gambusia affinis* (Baird and Girard). Arch. Environ. Contam. Toxicol. **18**: 819–825.

Norstrom, R.J., McKinnon, A.E., and deFreitas, A.S.W. 1976. A bioenergetics-based model for pollutant accumulation by fish. Simulation of PCB and methyl mercury residue levels in Ottawa River yellow perch (*Perca flavescans*). J. Fish. Res. Board Can. **33**: 248–267.

Parks, J.W., Curry, C., Romani, D., and Russell, D.D. 1991. Young northern pike, yellow perch and crayfish as bioindicators in a mercury contaminated watercourse. Environ. Monit. Assess. **16**: 39–73.

Parks, J.W., Craig, P.C., and Ozburn, G.W. 1994. Relationships between mercury concentrations in walleye (*Stizostedion vitreum*) and northern pike (*Esox lucius*): implications for modelling and biomonitoring. Can. J. Fish. Aquat. Sci. **51**: 2090–2104.

Phillips, G.R., and Buhler, D.R. 1978. The relative contributions of methyl mercury from food or water to rainbow trout (*Salmo gairdneri*) in a controlled laboratory environment. Trans. Am. Fish. Soc. **107**: 853–861.

Phillips, G.R., Lenhart, T.E., and Gregory, R.W. 1980. Relation between trophic position and mercury accumulation among fishes from the Tongue River Reservoir, Montana. Environ. Res. **22**: 73–80.

Ponce R.A., and Bloom, N.S. 1991. Effect of pH on the bioaccumulation of low level, dissolved methylmercury by rainbow trout (*Oncorhynchus mykiss*). Water Air Soil Pollut. **56**: 631–640.

Post, J.R. 1984. Planktivorous fish and the structure of pelagic plankton communities. M.Sc. thesis, York University, Toronto, Ont.

Post, J.R. 1990. Metabolic allometry of larval and juvenile yellow perch (*Perca flavescens*): in situ estimates and bioenergetic models. Can. J. Fish. Aquat. Sci. **47**: 554–560.

Post, J.R., and McQueen, D.J. 1994. Variability in first year growth of yellow perch (*Perca flavescens*): predictions from a simple model, observations and an experiment. Can. J. Fish. Aquat. Sci. **51**: 2501–2512.

Ramsey, D.E., and Ramlal, P.S. 1987*a*. Measurements of mercury methylation balance in relation to concentrations of total mercury

in northern Manitoba reservoirs and their use in predicting the duration of fish mercury problems in new reservoirs. *In* Technical appendices to the summary report, Canada–Manitoba agreement on the study and monitoring of mercury in the Churchill River Diversion. Vol. 3. Chap. 12. Government of Canada, Ottawa, Ont., and Government of Manitoba, Winnipeg, Man.

Ramsey, D.E., and Ramlal, P.S. 1987*b*. Measurements of rates of production and degradation of methyl mercury and concentrations of total mercury in Southern Indian Lake, Cedar Lake, and Granville Lake, Manitoba: results of a survey conducted in July and August, 1985. *In* Technical appendices to the summary report, Canada–Manitoba agreement on the study and monitoring of mercury in the Churchill River Diversion. Vol. 3. Chap. 13. Government of Canada, Ottawa, Ont., and Government of Manitoba, Winnipeg, Man.

Rasmussen, J.B., Rowan, D.J., Lean, D.R.S., and Carey, J.H. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Can. J. Fish. Aquat. Sci. **47**: 2030–2038.

Rice, J.A., and Cochran, P.A. 1984. Independent evaluation of a bioenergetics model for largemouth bass. Ecology, **65**: 732–739.

Richman, L.A., Wren, C.D., and Stokes, P.M. 1988. Facts and fallacies concerning mercury uptake by fish in acid stressed lakes (review article). Water Air Soil Pollut. **37**: 465–473.

Riisgard, H.U., and Hansen, S. 1990. Biomagnification of mercury in a marine grazing food-chain: algal cells *Phaeodactylum tricornutum*, mussels *Mytilus edulis* and flounders *Platichthys flesus* studied by means of a stepwise reduction – CVAA method. Mar. Ecol. Prog. Ser. **62**: 259–270.

Rodgers, D.W., and Beamish, F.W.H. 1981. Uptake of waterborne methyl mercury by rainbow trout (*Salmo gairdneri*) in relation to oxygen consumption and methyl mercury concentration. Can. J. Fish. Aquat. Sci. **38**: 1309–1315.

Rodgers, D.W., and Beamish, F.W.H. 1983. Water quality modifies uptake of waterborne methylmercury by rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. **40**: 824–828.

Rodgers, D.W., and Quadri, S.U. 1982. Growth and mercury accumulation in yearling yellow perch, *Perca flavescens*, in the Ottawa River, Ontario. Environ. Biol. Fishes, **7**: 377–383.

Rudd, J.W.M., and Turner, M.A. 1983. The English–Wabigoon River system. V. Mercury and selenium bioaccumulation as a function of aquatic primary productivity. Can. J. Fish. Aquat. Sci. **40**: 2251–2259.

Rudd, J.W.M., Furutani, A., and Turner, M.A. 1980. Mercury methylation by fish intestinal contents. Appl. Environ. Microbiol. **40**: 777–782.

Rudd, J.W.M, Turner, M.A., Furutani, A., Swick, A.L., and Townsend, B.E. 1983. The English–Wabigoon river system. I. A synthesis of recent research with a view towards mercury amelioration. Can. J. Fish. Aquat. Sci. **40**: 2206–2217.

Sharpe, M.A., deFreitas, A.S.W., and McKinnon, A.E. 1977. The effect of body size on methyl mercury clearance by goldfish (*Carassius auratus*). Environ. Biol. Fishes, **2**: 177–183.

Sorensen, E.M.B. 1991. Mercury. *In* Metal poisoning in fish. CRC Press, Inc., Boca Raton, Fla. pp. 285–331.

Sprules, W.G., Holtby, L.B., and Griggs, G. 1981. A microcomputer model measuring device for biological research. Can. J. Zool. **59**: 1611–1614.

Spry, D.J., and Wiener, J.G. 1991. Metal bioavailability and toxicity to fish in low alkalinity lakes: a critical review. Environ. Pollut. **71**: 243–304.

Suns, K., Curry, C., and Russell, D. 1980. The effects of water quality and morphometric parameters on mercury uptake by yearling yellow perch. Ontario Ministry of the Environment, Rexdale, Ont.

Suns, K., Hitchin, G., Loescher, B., Pastorek, E., and Pearce, R. 1987. Metal accumulations in fishes from Muskoka–Haliburton lakes in Ontario (1978–1984). Ontario Ministry of the Environment, Rexdale, Ont.

Treasurer, J.W. 1989. Mortality and production of 0+ perch, *Perca fluviatilis* L., in two Scottish lakes. J. Fish Biol. **34**: 913–928.

Verta, M. 1990. Changes in fish mercury concentrations in an intensively fished lake. Can. J. Fish. Aquat. Sci. **47**: 1888–1897.

Watras, C.J., and Bloom, N.S. 1992. Mercury and methyl mercury in individual zooplankton: implications for bioaccumulation. Limnol. Oceanogr. **37**: 1313–1318.

Wiener, J.G., Fitzgerald, W.F., Watras, C.J., and Rada, R.G. 1990. Partitioning and bioavailability of mercury in an experimentally acidified Wisconsin lake. Environ. Toxicol. Chem. **9**: 909–918.

Winfrey, M.R., and Rudd, J.W.M. 1990. Environmental factors affecting the formation of methylmercury in low pH lakes. Environ. Toxicol. Chem. **9**: 853–869.

Wren, C.D., and MacCrimmon, H.R. 1986. Comparative bioaccumulation of mercury in two adjacent freshwater ecosystems. Water Res. **20**: 763–769.

Wren, C.D., and Stephenson, G.L. 1991. The effect of acidification on the accumulation and toxicity of metals to freshwater invertebrates. Environ. Pollut. **71**: 205–241.

The influence of temperature on mRNA levels for muscle contractile protein and a proto-oncogene associated with cell division in Atlantic salmon (*Salmo salar* L.)

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Abstract: In Atlantic salmon (*Salmo salar* L.) embryos, an increase in temperature from 5 to 8 or 11°C causes, by the time of hatching, an increase in myofibrillar protein content and a reduction in nuclear proliferation when compared with embryos raised at lower temperatures. This implies a shift in emphasis during embryonic development from nuclear hyperplasia to contractile protein accretion at higher temperatures. The steady-state mRNA levels of proteins indicative of these processes were investigated. The proteins were actin, as a measure for myofibrillar protein, and cellular *myc*, as a measure for nuclear proliferation. Additionally a probe for myosin heavy chain was used. The mRNA levels for myofibrillar protein were not increased at the higher temperature, thus suggesting temperature-dependent translational or post-translational control. Actin and myosin heavy chain mRNA responses differed, supporting the existence of temperature-dependent myosin heavy chain isoforms. The presence of c-*myc* mRNA in embryonic Atlantic salmon was shown. The mRNA level of this nuclear proliferation marker was decreased at higher temperatures and could be related to histological data on nuclear numbers in embryonic salmon muscle.

Résumé : Chez les embryons de saumon atlantique (*Salmo salar*), une augmentation de la température de 5 à 8 ou 11°C cause, au moment de l'éclosion, une augmentation de la teneur en protéines myofibrillaires et une réduction de la prolifération nucléaire par rapport aux valeurs observées chez des embryons élevés à des températures inférieures. Cela indique qu'au cours du développement de l'embryon, l'accrétion des protéines contractiles devient plus importante que l'hyperplasie nucléaire aux températures plus élevées. On a étudié les teneurs à l'équilibre en mRNA des protéines caractérisant ces processus. Les protéines étaient l'actine, utilisée pour mesurer les protéines myofibrillaires, et le *myc* cellulaire, qui sert à mesurer la prolifération nucléaire. On a également utilisé une sonde sensible à la chaîne à poids moléculaire élevé de la myosine. Les teneurs en mRNA pour les protéines myofibrillaires n'augmentaient pas en fonction de la température, ce qui suggère un mécanisme de régulation dépendant de la température pendant ou après la translation. Les réponses de l'actine et de la chaîne à poids moléculaire élevé de la myosine différaient, ce qui appuie l'hypothèse de l'existence d'isoformes de la chaîne à poids moléculaire élevé de la myosine dépendant de la température. On a démontré la présence de mRNA dans le c-*myc* des embryons de saumon atlantique. La teneur en mRNA de ce marqueur de la prolifération nucléaire diminuait aux températures plus élevées et pourrait être reliée à des données histologiques sur les valeurs nucléaires mesurées dans les tissus musculaires des embryons de saumons.
[Traduit par la Rédaction]

Introduction

Fish are ectothermic animals and embryonic muscle growth, like many other developmental processes such as those concerned with meristic variation and organ growth, is influenced by temperature (Blaxter 1988; Hayes et al. 1953; Johnston 1993). Muscle growth and development occur by an increase in the size of individual fibres (muscle fibre hypertrophy) and by the addition of new muscle fibres (muscle fibre hyperplasia). Both processes necessitate the addition of nuclei to muscle tissue (Cardasis and Cooper 1975; Enesco and Puddy 1964; Nag and Nursall 1972) and, in fish, both play an important role throughout a large proportion of the animals' lives (Stickland 1983; Weatherly and Gill 1985; Vegetti et al. 1990).

In Atlantic salmon (*Salmo salar* L.) temperature exerts a dramatic influence on the muscle cellularity in embryonic stages; temperatures of only 3–6°C above ambient lead to a greater increase in hypertrophic growth relative to muscle fiber hyperplasia (Stickland et al. 1988; Usher et al. 1994). In other words, embryos at a developmental stage around the time of hatching have, in cross section, larger but fewer muscle fibres at the higher temperatures without overall muscle cross-sectional area being affected. Usher et al. (1994) found that this is associated, in Atlantic salmon embryos, with higher myofibrillar protein accretion, as determined by proportional myofibrillar area in white muscle fibers, and a lower number of muscle nuclei per cross-sectional area by the time of hatching at higher temperatures. The mechanism for this change in

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emphasis from nuclear proliferation to myofibrillar protein accretion is unknown.

Fish myosin heavy chain and actin DNA sequences have recently become available (e.g., Gerlach et al. 1990). This represents an opportunity to directly analyze muscle contractile protein mRNA levels whereas previous investigations in fish larvae have mainly focused on total body RNA and protein levels to study growth (e.g., Buckley 1984; Hovenkamp and Witte 1991; Westerman and Holt 1988; Mathers et al. 1993). The effect of temperature on contractile protein mRNA levels combined with the known temperature response of contractile protein accretion make it possible to judge whether regulation takes place pretranslationally, or alternatively affects translation or protein stability. One aim of this study was therefore to gain information on the regulatory steps in the temperature response of contractile protein synthesis in embryonic salmon.

The origin of new muscle nuclei in fish is still unclear. Myoblastic cells (sometimes also referred to as presumptive satellite cells (Johnston 1993)) or satellite cells are generally regarded as their origin (Campion 1984). However, in carp (*Cyprinus carpio* L.) there is a lack of proliferating satellite cells (Koumans et al. 1991, 1994), and amitotic division has been proposed as a mechanism in amphibia (Boujelida and Munk 1987). Early response genes such as cellular *myc* (c-*myc*) are associated with cell division (Cole 1986; Littlewood and Evan 1990). This proto-oncogene has been found in rainbow trout (*Oncorhynchus mykiss* Walbaum), a salmonid, and its sequence is available (van Beneden et al. 1986). A further aim of this study was therefore to assess the presence of c-*myc* in Atlantic salmon and to investigate how it relates to the nuclear proliferation patterns found at different temperatures.

Materials and methods

Animals

The Atlantic salmon embryos were derived from a single adult pairing from the same stock as the fish used by Usher et al. (1994). After fertilization the eggs were divided into three groups and kept either at ambient river temperature or in thermostat-regulated water at 8 or 11°C at the SOAFD Freshwater Fisheries Laboratory, Almondbank, Perthshire, Scotland. The ambient river temperature corresponded to an average daily temperature of 3.4°C.

At Gorodilov (1983) stages 25, 27, 29, 31, and 33 the embryos were sent to London by rail in water of the appropriate temperature insulated in polystyrene containers. The eggs were then kept in incubators at 5°C for the ambient river temperature group or at 8 and 11°C, as appropriate, for at least 24 h to let the embryos recover from any fluctuation in temperature during transport.

At the appropriate sampling time the salmon eggs were dechorionated if necessary, the embryos were killed by decapitation, and the yolk sac was removed so that the remaining tissue consisted mainly of muscle. The tissue was stored in liquid nitrogen until further use. Because of the small size of the fish, 10 to 15 embryos were pooled per sample.

RNA isolation and fractionation

The RNA was isolated as described by Chomczynski and Sacchi (1987). The amount of RNA present was estimated by determining the optical density of the solution at 260 nm (OD_{260}) and purity was estimated from the ratio OD_{260}/OD_{280}. The RNA was then immediately fractionated on a denaturing agarose gel and blotted according to the modified method of Fourney et al. (1988). Briefly, 20 µg of total RNA were made up to a volume of 15 µL with deionized H_2O treated with diethyl pyrocarbonate and then 15 µL of loading buffer without bromophenol blue was added. Ethidium bromide was added to a 1% agarose gel at a final concentration of 0.01%. The gel was run at 4°C for 20 h with a voltage of 30 V and then photographed on a UV transilluminator. Finally, the RNA was blotted onto nylon membrane (Hybond N, Amersham) using standard methods (Sambrook et al. 1989). The RNA was fixed to the membrane by baking at 80°C for 2 h.

DNA probes

For muscle contractile protein a carp actin probe, specific for both alpha and beta actins (FGA101, Gerlach et al. 1990), and a carp myosin heavy chain (MyHC) probe, corresponding to exon 40 (FG2Ex40), were used and labelled with ^{32}P by random priming (Amersham Multiprime DNA labelling system). The actin probe had been shown to correspond to a highly conserved region in its respective gene (Gerlach et al. 1990). The MyHC probe corresponded to exon 40 in the relatively conserved rod region.

An oligonucleotide (48-mer) corresponding to a conserved region, nucleotide 778 to nucleotide 825, of the rainbow trout cellular c-*myc* gene (van Beneden et al. 1986) served as a probe for c-*myc*. The probe was 3′-end labelled with ^{32}P as described (Titus 1991) using terminal transferase (enzyme and buffer from Pharmacia).

Northern hybridization

The Northern hybridization was carried out as recommended by the membrane manufacturer (Amersham). Briefly, the hybridization took place at 42°C in 5× SSPE buffer containing 5× Denhardt's solution and 0.5% sodium lauryl sulphate (SDS) at a final concentration of 50% formamide. Washes were carried out under low stringency conditions, i.e., at 65°C and with 1× SSPE, 0.1% SDS buffer, with the exception of the hybridization reaction with the c-*myc* probe, which was washed at 55°C. After autoradiography the membrane was stripped of bound probe twice by pouring on boiling SDS solution (0.1%) and then reused for further experiments.

Evaluation

The washed blots were analyzed by autoradiography at –70°C employing intensifying screens (Amersham). To be able to quantify the results the films were preflashed to give a linear response to the amount of radioactivity present.

The autoradiographs were analyzed using a Seescan image analyzer to determine the integral optical density for each sample and probe. The results were normalized against the amounts of ribosomal RNA (rRNA) present, as determined from the amount of ethidium bromide bound to the nucleic acid, to compensate for possible pipetting errors. Ribosomal RNA represents the bulk of RNA present in total RNA (Young 1976) and therefore is a good measure for the amount of the latter. It is routinely used as an internal control in Northern blotting (see for example Hesketh et al. 1992; Iwaki et al. 1990). For the measurements of rRNA a negative image of the UV-transilluminated agarose gel was produced from a photograph and analyzed in the same way as the autoradiographs. The values for both species (28S and 18S) of rRNA were combined. The size of the RNA species identified was estimated by comparison with the migration distances of the 28S and 18S rRNA.

Statistics

The data were analyzed by two-way analysis of variance and the means were compared by the Newman–Keuls method (Snedecor and Cochran 1969).

Results

Northern hybridization

Figure 1 shows the autoradiographs for the various probes

used and the agarose gel stained with ethidium bromide. As can be seen, clear specific bands of the appropriate sizes were detected. There was almost no variation in the amount of total RNA pipetted onto the gel (Fig. 1D).

The cDNA specific for carp muscle contractile protein transcript hybridized to the salmon RNA. The probes bound only to bands of the expected size and no nonspecific binding was observed. With progressing development the relative amount of specific messenger RNA (mRNA) was found to increase. This was more pronounced for actin ($p < 0.001$) than for MyHC ($p = 0.029$) (Figs. 2A and 2B). At stage 31 (around the time of hatching) no such increase of contractile protein mRNA with increasing developmental age was observed at ambient temperature and 8°C. For both actin and MyHC a statistically highly significant ($p < 0.001$) decrease in mRNA steady state levels was observed at 8 and 11°C compared with at ambient temperature. However, no statistically significant difference in contractile protein mRNA levels was found between these two elevated temperatures. A comparison between the mRNA levels for MyHC and actin shows that there was proportionately more signal for MyHC than for the actin probe the lower the temperature ($p < 0.001$), whereas there was relatively little to no change with development ($p = 0.103$) (Fig. 2C).

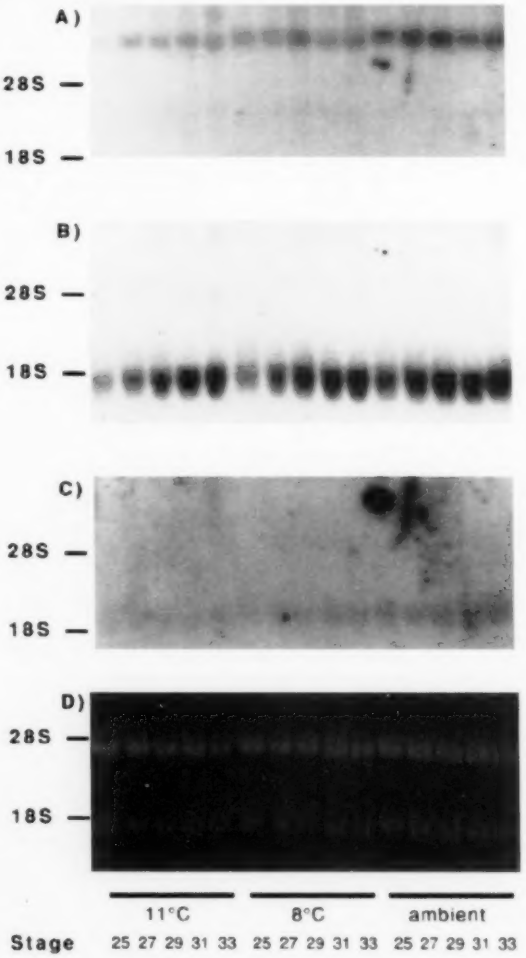
The c-*myc* transcript was clearly present in the embryos with lower temperature fish exhibiting a higher steady state level ($p = 0.002$) (Fig. 2D). Embryos raised at 11 and 8°C behaved in a very similar way, with the 8°C fish showing only a marginally stronger signal at stage 33 (not significant). At ambient temperature an increase with development appeared to be present whereas both 8 and 11°C mRNA levels remained relatively constant.

The proportionate increase of actin mRNA in comparison with c-*myc* mRNA was calculated as a ratio. The result is shown in Fig. 2E. More c-*myc* relative to actin mRNA was present at the ambient temperature ($p < 0.05$). No statistically significant change with development was found ($p = 0.789$).

Discussion

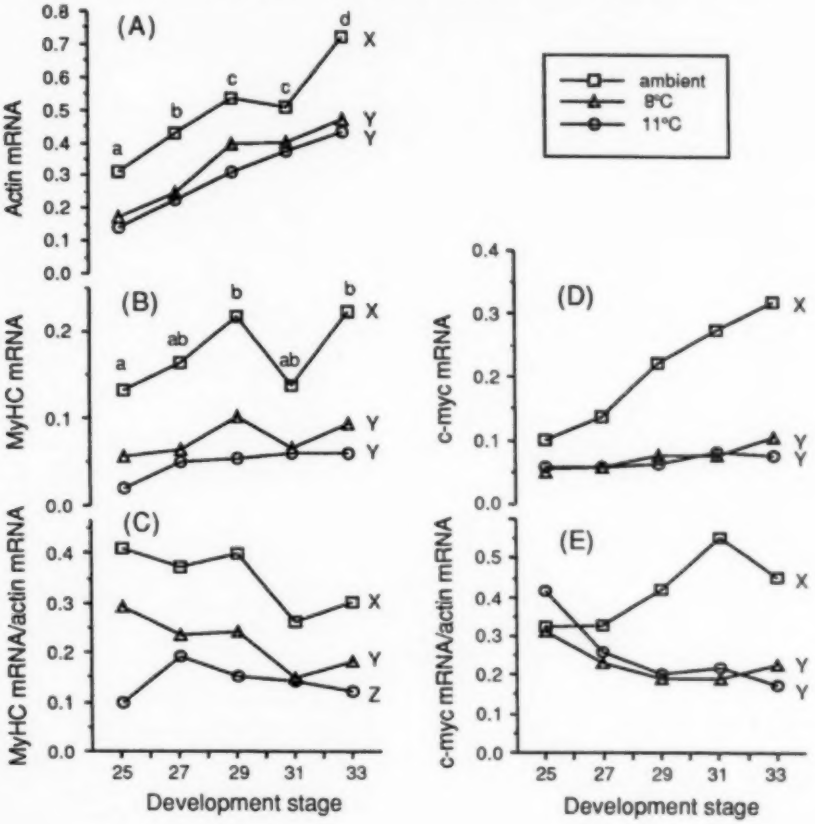
Both carp probes (actin and MyHC) were found to cross-react with the appropriate salmon RNA species. In the case of the actin probe, cross-reaction was not surprising because only two striated muscle actin isoforms are known (Buckingham 1985) and the actin probe used has been shown to correspond to a highly conserved region within the molecule (Gerlach et al. 1990). In Fig. 2B two actin bands appear to be present. The size difference between these bands is smaller than would be expected between α-actin and cytoskeletal actin, and also smaller than the difference found for α- and β-actin in carp by Gerlach et al. (1990). No heart tissue was present in the samples and the two bands were thus combined and assumed to represent myofibrillar actin. The data obtained for actin should therefore represent a good measure for muscle contractile protein mRNA. As has been found in mammals (Buckingham 1985) the mRNA levels increased with development at all three temperature regimes used. Interestingly, the amount of contractile protein mRNA remained at the same level for 8°C and ambient river temperature at stage 31 when compared with stage 29. In the salmon embryos used, stage 31 coincided with the hatching period. Hatching has been associated with a reduction in growth and this may be responsible for this particular finding (Rombough 1988).

Fig. 1. Autoradiographs of a nylon membrane probed with DNA specific for (A) myosin heavy chain (exon 40) and (B) actin under low stringency conditions (1× SSC, 0.1% SDS, 65°C), and (C) c-*myc* (1× SSC, 0.1% SDS, 55°C; note the c-*myc* specific bands near the position of the 18S rRNA). In (D) the level of ethidium bromide bound to the ribosomal RNA in the gel used for the above blots is shown. Twenty micrograms of total RNA was applied to each lane. The positions of the 28S and 18S ribosomal mRNAs are shown. From left to right, developmental stages 25, 27, 29, 31, and 33 were applied for each temperature.



Embryos reared at a temperature above the ambient river temperature did not exhibit higher contractile protein mRNA levels; on the contrary, a lower level of steady state mRNA for actin or MyHC was found at the higher temperatures for all developmental stages investigated. Usher et al. (1994) found higher protein accretion at an increased temperature (11°C against 5 or 8°C) for the same stock of fish and it would thus seem that this higher myofibrillar protein content is effected

Fig. 2. (A) Actin, (B) myosin heavy chain (MyHC), and (D) c-*myc* steady state mRNA levels for the five different developmental stages and the three temperatures employed. The amounts are expressed as relative optical density on autoradiographs normalized for the amount of ribosomal RNA present. In C and E, the amounts of MyHC mRNA (from B) and c-*myc* (from D), respectively, are shown relative to actin mRNA (from A). A statistically significant difference ($p < 0.05$) is indicated by differing letters (for temperature, uppercase letters; for development, lowercase letters). Ambient temperature averaged 3.4°C over the entire experimental period.



by translational or post-translational control rather than through upregulation of transcription or higher mRNA stability. Evidence for translational control has been found, for example, in the stimulation of hypertrophy in rat gastrocnemius muscle by clenbuterol (Hesketh et al. 1992), or in muscle protein synthesis in cod in response to different ration sizes (von der Decken and Lied 1989). A variation in protein stability, i.e., post-translational control, is, however, also a possible explanation for the differences in protein accretion observed in embryonic salmon. No statistically significant difference in the contractile protein mRNA levels between 8 and 11°C was found in this study, whereas the myofibrillar contents had been found to be different (Usher et al. 1994). This again suggests that the control of contractile protein accretion does not take place before translation.

The MyHC transcript, which was investigated as a second contractile protein mRNA, exhibited a result similar to that found for actin. The embryos grown at ambient river temperature showed the highest level of MyHC mRNA compared with those grown at 11 or 8°C. When the results are directly compared with the results for actin, a higher MyHC/actin ratio is obtained the lower the experimental temperature. Bearing in mind that the number of MyHC isoforms is substantially larger than for actin, this result may be interpreted in the following way. In mammals various developmental isoforms are present (Buckingham 1985) and in carp a minimum of 28 MyHC genes seem to be present and isoform switching may be involved in temperature acclimation (Gerlach et al. 1990). The MyHC probe used corresponds to a region in exon 40 of the gene, which is part of the relatively (but not fully) conserved rod region. The present result may arise because the probe did not, at least in the salmon, hybridize to all isoforms, which would have resulted in a similar MyHC/actin ratio regardless of the temperature treatment. Perhaps, for this reason, the embryonic salmon, like the carp, exhibits an isoform switch at different temperatures. Differences in MyHC isoform depending on water temperature have been reported in a number of studies (e.g., cod: Gerlach et al. 1990; Hwang et al. 1990; Arctic

char: Martinez and Patterson 1992). Alternatively, different translation rates or protein stabilities for actin and MyHC could lead to the observed result. The mRNA data found would then simply represent different transcription rates or mRNA stabilities for actin and total MyHC. As is discussed by Lowery and Somero (1990) for starvation in *Paralabrax nebulifer*, complex changes in ribosomal activity and capacity combined with changes in protein turnover rates can differentially affect protein levels. Unfortunately no salmon probes for the 3′ untranslated region of the MyHC gene, which would make it possible to clarify the situation, are available as yet.

The expression of c-*myc* has been found to be associated with the entry of cells into the cell cycle and correlates with the rate of cell proliferation (reviewed in Cole 1986 and Littlewood and Evan 1990). Furthermore, the amount of c-*myc* protein present directly correlates with mRNA levels (Hann and Eisenmann 1984) and its protein half-life does not change during growth (Persson et al. 1985). In muscle, c-*myc* expression declines with the onset of differentiation and plays a role in the control of proliferation and differentiation (reviewed in Olson 1992). Both c-*myc* mRNA and protein have a very short half-life and have to be expressed throughout the cell cycle (Littlewood and Evan 1990). Additionally c-*myc* mRNA levels have been shown to fall with the replacement of hyperplasia with hypertrophy in mouse forebrain, hindbrain, and kidney (Zimmerman et al. 1986). The c-*myc* transcript was clearly present and more prevalent at the lower temperature, indicating a higher level of nuclear proliferation in this case. This reflects the result found for nuclear numbers at hatching for different temperatures in the same stock of fish (Usher et al. 1994).

A comparison of the steady state mRNA levels for actin as a measure for contractile muscle protein and c-*myc* as a measure for nuclear proliferation showed a higher c-*myc*/actin ratio at ambient temperature than at 11°C. This difference appears to be present only from stage 29 onwards, but no statistical difference between the developmental stages was found. The result would be consistent with the histological findings of Usher et al. (1994) who found no difference in nuclear numbers and myofibrillar area at stage 25 but a significant difference at stage 33. However, it should be emphasized that the actin mRNA levels do not relate directly to the contractile protein levels.

In summary, this study has demonstrated a reduction in contractile protein mRNA (actin and MyHC) at increased temperatures and suggests that contractile protein accretion in embryonic Atlantic salmon is achieved by translational or post-translational control rather than at the transcriptional or mRNA stability level. The presence of c-*myc* mRNA was demonstrated and the results show a relative reduction in its level with an increase in temperature.

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References

Blaxter, J.H.S. 1988. Pattern and variety in development. *Edited by* W.S. Hoar and D.J. Randall. Fish physiology. Vol. XI. Part A. Academic Press, London. pp. 1–58.

Boujelida, H., and Munk, L. 1987. Multinucleation during myogenesis of the myotome of *Xenopus laevis*: a qualitative study. Development (Cambridge), **101**: 583–590.

Buckingham, M. 1985. Actin and myosin multigene families: their expression during the formation of skeletal muscle. Essays Biochem. **20**: 77–109.

Buckley, L.J. 1984. RNA–DNA ratio: an index of larval fish growth in the sea. Mar. Biol. (Berlin), **80**: 291–298.

Campion, D.R. 1984. The muscle satellite cell: a review. Int. Rev. Cytol. **87**: 225–251.

Cardasis, C.E., and Cooper, G.W. 1975. An analysis of nuclear numbers on individual muscle fibres during differentiation and growth: a satellite cell – muscle fibre growth unit. J. Exp. Zool. **191**: 347–358.

Chomczynski, P., and Sacchi, N. 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162**: 156–159.

Cole, M.D. 1986. The myc oncogene: its role in transformation and differentiation. Annu. Rev. Genet. **20**: 361–384.

Enesco, M., and Puddy, D. 1964. Increase in the number of nuclei and weight in skeletal muscle of rats of various ages. Am. J. Anat. **114**: 235–244.

Fourney, R.M., Miyakoshi, J., Day, R.S., III, and Paterson, M.C. 1988. Northern blotting: efficient RNA staining and transfer. Betheseda Res. Lab. Focus, **10**: 5–6.

Gerlach, G.-F., Turay, L., Malik, K.T.A., Lida, J., Scutt, A., and Goldspink, G. 1990. Mechanisms of temperature acclimation in the carp; a molecular biology approach. Am. J. Physiol. **259**: R237–R244.

Gorodilov, Yu.N. 1983. Stadii embryonalnogo razvitiya atlanticheskogo lososya *Salmo salar* L. II. Opisaniye i khronologia. Sb. Nauchn. Tr. Gosniorkh. **200**: 107–126. (Translated by J.E. Thorpe. Scott. Fish. Res. Transl. **19**: 1–34.)

Hann, S.R., and Eisenmann, R.N. 1984. Proteins encoded by the human c-*myc* oncogene: differential expression in neoplastic cells. Mol. Cell. Biol. **4**: 2486–2497.

Hayes, F.R., Pelluet, D., and Gorham, E. 1953. Some effects of temperature on the embryonic development of the salmon (*Salmo salar*). Can. J. Zool. **31**: 41–52.

Hesketh, J.E., Campbell, G.P., Lobley, G.E., Maltin, C.A., Acamovic, F., and Palmer, R.M. 1992. Stimulation of actin and myosin synthesis in rat gastrocnemius muscle by clenbuterol; evidence for translational control. Comp. Biochem. Physiol. C, **102**: 23–27.

Hovenkamp, F., and Witte, J.I.J. 1991. Growth, otolith growth and RNA/DNA ratios of larval plaice, *Pleuronectes platessa*, in the North Sea, 1987 to 1988. Mar. Ecol. Prog. Ser. **70**: 105–116.

Hwang, G.C., Watabe, S., and Hashimoto, K. 1990. Changes in carp myosin ATPase induced by temperature acclimation. J. Comp. Physiol. B, **160**: 233–239.

Iwaki, K., Sukhatme, V.P., Shubeita, H.E., and Chien, K.R. 1990. α- and β-adrenergic stimulation includes distinct patterns of immediate early gene expression in neonatal rat myocardial cells. J. Biol. Chem. **265**: 13 809 – 13 817.

Johnston, I.A. 1993. Temperature influences muscle differentiation and the relative timing of organogenesis in herring (*Clupea harengus*) larvae. Mar. Biol. (Berlin), **116**: 363–379.

Koumans, J.T.M., Akster, H.A., Booms, R.G.H., Lemmens, C.J.J., and Osse, J.W.M. 1991. Numbers of myosatellite cells in white

axial muscle of growing fish: *Cyprinus carpio* L. (Teleostei). Am. J. Anat. **192**: 418–424.

Koumans, J.T.M., Akster, H.A., Booms, G.H.R., and Osse, J.W.M. 1994. Numbers of muscle nuclei and myosatellite cell nuclei in red and white axial muscle during growth of the carp (*Cyprinus carpio* L.). J. Fish Biol. **44**: 391–408.

Littlewood, T.D., and Evan, G.I. 1990. The role of *myc* oncogenes in cell growth and differentiation. Adv. Dent. Res. **4**: 69–79.

Lowery, M.S., and Somero, G.N. 1990. Starvation effects on protein synthesis in red and white muscle of the barred sand bass, *Pleuronectes nebulifer*. Physiol. Zool. **63**: 630–648.

Martinez, I., and Petterson, G.W. 1992 Temperature-induced precocious transitions of myosin heavy chain isoforms on the white muscle of the Arctic charr *Salvelinus alpinus* (L.). Basic Appl. Myol. **2**: 89–95.

Mathers, E.M., Houlihan, D.F., McCarthy, I.D., and Burren, L.J. 1993. Rates of growth and protein synthesis correlated with nucleic acid content in fry of rainbow trout, *Oncorhynchus mykiss*; effects of age and temperature. J. Fish Biol. **43**: 245–263.

Nag, A.C., and Nursall, J.R. 1972. Histogenesis of white and red muscle fibers of trunk muscles of a fish *Salmo gairdneri*. Cytobios, **6**: 227–246.

Olson, E.N. 1992. Interplay between proliferation and differentiation within the myogenic lineage. Dev. Biol. **154** : 261–272.

Persson, H., Gray, H.E., and Godeau, F. 1985. Growth dependent synthesis of c-*myc*-encoded proteins: early stimulation by serum factors in synchronised mouse 3T3 cells. Mol. Cell. Biol. **5**: 2903–2912.

Rombough, P.J. 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. *In* Fish physiology. Vol. XI. Part A. *Edited by* W.S. Hoar and D.J. Randall. Academic Press, London. pp. 59–161.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. pp. 7.46–7.50.

Snedecor, G.W., and Cochran, W.G. 1969. Statistical methods. 6th ed. The Iowa State University Press, Ames, Iowa. pp. 299–338.

Stickland, N.C. 1983. Growth and development of muscle fibres in the rainbow trout (*Salmo gairdneri*). J. Anat. **137**: 323–333.

Stickland, N.C., White, R.N., Mescall, P.E., Crook, A.R., and Thorpe, J.E. 1988. The effect of temperature on myogenesis in embryonic development of the Atlantic salmon (*Salmo salar* L.). Anat. Embryol. **178**: 253–257.

Titus, D.E. 1991. Promega protocols and applications guide. Promega Corporation, Madison, Wis. p. 148.

Usher, M.L., Stickland, N.C., and Thorpe, J.E. 1994. Muscle development in Atlantic salmon (*Salmo salar*) embryos and the effect of temperature on muscle cellularity. J. Fish Biol. **44**: 953–964.

van Beneden, R.J., Watson, D.K., Chen, T.T., Lautenberger, J.A., and Papas, T.S. 1986. Cellular *myc* (c-*myc*) in fish (rainbow trout): its relationship to other vertebrate *myc* genes and to the transforming genes of the MC29 family of viruses. Proc. Natl. Acad. Sci. U.S.A. **83**: 3698–3702.

Vegetti, A., Mascarello, F., Scapolo, P.A., and Rowlerson, A. 1990. Hyperplastic and hypertrophic growth of lateral muscle in *Dicentrarchus labrax* (L.). An ultrastructural and morphometric study. Anat. Embryol. **182**: 1–10.

von der Decken, A., and Lied, E. 1989. Myosin heavy chain synthesis in white trunk muscle of cod (*Gadus morhua*) fed different ration sizes. Fish Physiol. Biochem. **6**: 333–340.

Weatherly, A.H., and Gill, H.S. 1985. Dynamics of increase in muscle fibers in fishes in relation to size and growth. Experientia, **41**: 353–354.

Westerman, M.E., and Holt, G.J. 1988. The RNA–DNA ratio: measurement of nucleic acids in larval *Sciaenops ocellatus*. Contrib. Mar. Sci. **30**(Suppl.): 479–485.

Young, V.R. 1976. The role of skeletal and cardiac muscle in the regulation of protein metabolism. *In* Mammalian protein metabolism. Vol. 4. *Edited by* H.N. Munro. Academic Press, London. pp. 586–674.

Zimmerman, K.A., Yancopoulos, G.D., Collum, R.G., Smith, R.K., Kohl, N.E., Denis, K.A., Nau, M.M., White, O.N., Toran-Allerand, D., Gee, C.E., Minna, J.D., and Alt, F.W. 1986. Differential expression of *myc* family genes during murine development. Nature (London), **319**: 780–783.

Bayesian fishery stock assessment and decision making using adaptive importance sampling

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Abstract: Bayesian posterior distributions of multidimensional parameters of a surplus-production model are obtained by adaptive importance sampling (AIS) and sampling – importance – resampling (SIR). The main contribution of this work is the proposed combined use of both procedures (AIS-SIR) resulting in a stochastic simulation procedure that combines the virtues of a good importance function with the simplicity of the SIR algorithm. After describing the methods, I perform a Bayesian decision analysis for orange roughy (*Hoplostethus atlanticus*), show the good performance of the AIS-SIR procedure, and compare the results with the risk assessment of Francis (R.I.C.C. Francis. 1992. Can. J. Fish. Aquat. Sci. 49: 922–930).

Résumé : Par échantillonnage adaptatif en fonction de l'importance (AIS) et échantillonnage –rééchantillonnage en fonction de l'importance (SIR), on obtient des distributions bayesiennes postérieures pour les paramètres multidimensionnels d'un modèle surplus-production. La principale contribution innovatrice de ce travail est l'utilisation combinée proposée des ces deux techniques (AIS-SIR), qui donne une méthode de simulation stochastique combinant les avantages d'une fonction basée sur l'importance à la simplicité de l'algorithme SIR. Après avoir décrit les méthodes, j'effectue une analyse de décision pour l'hoplostète orange (*Hoplostethus atlanticus*), je démontre la bonne performance de la méthode AIS-SIR et je compare les résultats obtenus avec ceux de l'évaluation des risques de Francis (R.I.C.C. Francis. 1992. Can. J. Fish. Aquat. Sci. 49: 922–930).

Introduction

Fishery stock assessment involves many uncertainties that can make decisions about harvest difficult. Classical statistics, focused on parameter estimation and hypothesis testing, gives no direct advice on how to act in the face of a multitude of uncertain possibilities. In fact, some questions of practical interest such as "what is the probability of virgin biomass exceeding some given threshold?" are meaningless when using a classical approach where virgin biomass is typically an unknown (constant) parameter, and probabilities describe random quantities. To improve management, explicit recognition of both uncertainties and risks is essential (Hilborn et al. 1993*a*).

Bayesian statistics (Berger 1995) provides the theory to overcome these difficulties. It defines probability as a measurement of uncertainty (or relative credibility) and uses this definition consistently. Point estimates of classical statistics become probability densities in the Bayesian framework. This removes difficulties in answering the question about virgin biomass formulated above. Bayesian decision analysis goes a step further and helps us choose a best decision from a list of candidates.

Practical difficulties, induced by the complexity (nonlinearity and high dimensionality) of biologically meaningful models, have limited the implementation of the Bayesian approach until recently. Smith (1991) characterized the various stochastic simulation methods that can be used to perform the Bayesian computation.

This paper describes the application of adaptive importance sampling (AIS) with mixture models (West 1992, 1993) to perform the Bayesian calculations in a biomass dynamics model. This procedure is used as an extension of the simpler sampling – importance –resampling (SIR) algorithm (Rubin 1988) that has already been presented and applied in fisheries (e.g., Givens et al. 1993, 1994).

The presentation is organized as follows. I begin by describing basic importance sampling and show that the success of the method depends on the availability of a good importance function. To find such a function is the purpose of the adaptive procedure described next. A characterization of the SIR algorithm closes the section. I illustrate the method with Francis' (1992) data on orange roughy (*Hoplostethus atlanticus*) using a model for biomass dynamics. I then examine various features of the adaptive approach and propose its combined use with SIR. Finally, after defining utility, a Bayesian decision analysis is performed and the results compared with those obtained by Francis.

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Method

One expects uncertainties to change with the availability of relevant new information. Such changes have to translate into changes in the probabilities of competing hypotheses. If new information comes in the form of statistical data, then Bayes' theorem tells how the probabilities have to change. To state the theorem the following notation is used.

$\pi(\theta)$ is the probability of hypothesis θ prior to the observation of data. Such probability describes the relative credibility of $\theta \in S_\theta$

given all available background information. S_θ is the set of all possible hypotheses.

$L(\theta) = \Pr(\text{data}|\theta)$ is the conditional probability of the data given that θ is the true hypothesis. For fixed data it is called the likelihood function for θ.

$p(\theta|\text{data})$ is the probability of θ after accounting for the new evidence provided by the data.

Bayes' theorem says that the probability of θ after observing a given data set is proportional to the product of likelihood and prior. That is,

$$(1) \qquad p(\theta|\text{data}) \propto L(\theta) \times \pi(\theta)$$

The set of probabilities $p(\theta|\text{data})$ over S_θ is called posterior probability distribution (or simply posterior). It is the central element in Bayesian analysis.

The advantage of posterior distribution over point estimates is apparent when some best action has to be chosen from a list of possibilities. Let $U(\theta,a)$ denote the utility of action a when θ is the correct parameter. Bayesian decision theory says that the best action must maximize the expected posterior utility. If $E^{p(\theta)}[U(\theta,a)]$ denotes the expectation of $U(\theta,a)$ with respect to a probability distribution $p(\theta)$, the expected posterior utility of action a, $U(a)$, is defined by

$$(2) \qquad U(a) = E^{p(\theta|\text{data})}[U(\theta,a)]$$

It can be difficult, even impossible for high-dimensional θ and multimodal $p(\theta)$, to obtain an analytical solution of (2). Numerical procedures are often necessary. If a set $\{\theta_j; j = 1, \ldots, n\}$ of random draws from the posterior $p(\cdot)$ is available, then a Monte Carlo approximation for $U(a)$ is

$$(3) \qquad U(a) \approx \frac{1}{n}\sum_{j=1}^{n} U(\theta_j,a)$$

For general $p(\cdot)$ it can be hard to make such draws, limiting the practical implementation of the method. To overcome these hurdles, two distinct paths were attempted (Smith 1991): (*i*) Markov Chain methods (e.g., the Gibbs sampler) and (*ii*) importance sampling. According to Smith, provided that a good procedure can be found, it is likely that importance sampling is more efficient than the Markov Chain approach. This paper considers only importance sampling.

Basic importance sampling

In basic importance sampling (BIS) the density $p(\cdot)$ is replaced by the importance functions $g(\cdot)$, from which the random draws are made, producing two sets: $\Theta = \{\theta_j; j = 1, \ldots, n\}$ and $\Omega = \{\omega_j; j = 1, \ldots, n\}$: a collection of values for the parameter θ, with the corresponding probabilities given by ω. I will show that the Monte Carlo approximation of $U(a)$ is calculated from these sets, and given by

$$(4) \qquad U(a) \approx \frac{1}{n}\sum_{j=1}^{n} \omega_j U(\theta_j,a)$$

Thus, the key to this approach is the ability to find a suitable $g(\cdot)$. The adaptive construction of $g(\cdot)$ is described later. First I shall give the principles of BIS.

Suppose a situation where it is impractical to produce an independent random sequence from the posterior $p(\cdot)$, but the exact evaluation of the kernel $f(\theta) \propto L(\theta) \times \pi(\theta)$ is possible for any given θ (i.e., only keep those elements of L and π that depend on θ) and that some density $g(\cdot)$ close to $p(\cdot)$ is available for random draws. This is often the prior $\pi(\theta)$. Now imagine that we want to evaluate the expectation (with respect to $p(\cdot)$) of some given function $H(\cdot)$ (e.g., $U(a,\theta)$) using the Monte Carlo approximation.

If $E^p[\cdot]$ and $E^g[\cdot]$ denote the expectations under densities $p(\cdot)$ and $g(\cdot)$, respectively, then one can show (Kinas 1993, p. 18) that

$$(5) \qquad E^p[H(\cdot)] = \frac{E^g[H(\cdot)\omega(\cdot)]}{E^g[\omega(\cdot)]}$$

where $\omega(\theta) = f(\theta)/g(\theta)$.

We draw a random sample $\Theta = \{\theta_1, \ldots, \theta_n\}$ of size n from $g(\cdot)$ to approximate the expectations $E^g[\cdot]$ in (5). Instead of using $\omega(\theta_j)$ directly, it will be more convenient to use the corresponding set of normalized weights $\Omega = \{\omega_1, \ldots, \omega_n\}$, where ω_j is defined as $\omega_j = \omega(\theta_j)/k$ with $k = \sum_{j=1}^{n}\omega(\theta_j)$.

Some trivial algebra on expression (5) (Kinas 1993, pp. 18, 19) leads to the central result of the BIS procedure, which is to replace $p(\cdot)$ by a discrete approximation with mass function ω_j at θ_j. The expectation of $H(\cdot)$ is given as

$$(6) \qquad E^p[H(\cdot)] \approx \sum_{j=1}^{n} H(\theta_j)\omega_j$$

The description above assumes the existence of a good importance function. A suitable $g(\cdot)$ should make the approximation in (6) accurate for n as small as possible. These are sometimes competing goals, and different situations might need different solutions. Berger (1985, pp. 263–265) provides some guidelines for choosing an adequate $g(\cdot)$. Geweke (1989) gives mild conditions under which the numerical approximation (6) converges to the true value.

Although no general rule for choosing $g(\cdot)$ exists there are some desirable properties of a good importance function: (*i*) It should be easy to generate random draws from it, a requirement for the efficient use of Monte Carlo integration. (*ii*) It should not have sharper tails than $f(\cdot)$. Sharper tails would cause large values of $\omega(\theta_j)$ for outlying values of θ_j. The result would be increased instability in the estimates. (*iii*) It should mimic the true $f(\cdot)$. The goal is to avoid a large proportion of small $\omega(\theta_j)$. Such values have little effect on (6), reducing the efficiency of the method.

Adaptive importance sampling

It can be very difficult to find a good importance function through hard thinking only. For high-dimensional θ it can even be impossible. The prospect of constructing $g(\cdot)$ adaptively is therefore attractive. This was first considered by Kloeck and van Dijk (1978). Other methods were proposed by Naylor and Smith (1988) and West (1992, 1993). I use West's method that defines $g(\cdot)$ as a finite mixture of Student distributions. Three features justify my choice: (*i*) the capability of mixture models to emulate very irregular forms of distributions (Wolpert 1991), (*ii*) the practicality for high-dimensional θ, and (*iii*) the potential for automation.

AIS starts with some crude approximation for $p(\cdot)$ denoted by $g_0(\cdot)$ (the prior $\pi(\theta)$ is a natural candidate). Making n_0 random draws from $g_0(\cdot)$ produces the set of values $\Theta_0 = \{\theta_{0,j}; j = 1, \ldots, n_0\}$ and the associated (normalized) weights $\Omega_0 = \{\omega_{0,j}; j = 1, \ldots, n_0\}$.

Because many of the sample points $\theta_{0,j}$ will have negligible weights $\omega_{0,j}$ (i.e., small mass $p(\theta_{0,j})$ relative to $g_0(\theta_{0,j})$), this importance function is very inefficient computationally, requiring a huge sample size n_0, and may converge either slowly or not at all. The adaptive refinement consists in taking a small n_0 and, on the basis of the outcome, constructing the updated $g_1(\cdot)$. A new sample from $g_1(\cdot)$ should have a better match with $p(\cdot)$. The final importance function obtained by such an adaptive scheme is expected to display the desirable mimicry to $p(\cdot)$.

A summarized algorithm for the procedure is shown below. Assume θ has true density $p(\theta)$. Further assume that the kernel $f(\theta) \propto p(\theta)$ can be evaluated for any given θ. Proceed as follows.

- Step 1. Define an initial importance function $g_0(\cdot)$ and draw a random sample of size n_0 from it. (I will use the prior $\pi(\cdot)$.) Let $\Gamma_0 = \{g_0(\cdot), n_0, \Theta_0, \Omega_0\}$ represent the available quadruple after this initial step.
- Step 2. Compute the Monte Carlo estimate for the variance V_0, as the weighted sum

$$(7)\qquad V_0 = \sum_{j=1}^{n_0} \omega_{0,j}(\theta_{0,j} - \bar{\theta}_0)^2$$

where $\bar{\theta}_0 = \Sigma_{j=1}^{n_0} \omega_{0,j}\theta_{0,j}$ is the Monte Carlo mean.

• Step 3. Construct a revised importance function as the following weighted mixture of Student distributions:

$$(8)\qquad g_1(\theta) = \sum_{j=1}^{n_0} \omega_{0,j} T(\theta | k, \theta_{0,j}, h^2 V_0)$$

where $T(\cdot|k,m,M)$ denotes a heavy-tailed p-variate Student density with k degrees of freedom, mean m, variance M, and smoothing parameter h (see below).

• Step 4. Draw a larger sample $n_1 > n_0$ from $g_1(\theta)$ and replace Γ_0 by $\Gamma_1 = \{g_1(\cdot), n_1, \Theta_1, \Omega_1\}$.

• Step 5. Use diagnostics (see comments below) and either stop and base inference on Γ_1 or repeat steps 2 and 3 for a revised version Γ_2.

Some comments about the algorithm are necessary.

Student distribution

According to West (1993), this is an adequate choice provided that each component of θ is defined in the interval $(-\infty, +\infty)$. The bandwidth h that multiplies the variance V is a smoothing parameter as used in conventional kernel density estimation (Silverman 1986). Notice that there is no restriction on the dimensionality of θ. There is, however, a requirement to have each component of θ defined on the real line. This implies using transformations for parameters that do not satisfy this requirement. Finally, heavy tails are obtained from small values of degrees of freedom ($k < 10$). Algorithms to perform random draws from uni- and multi-dimensional Student densities can be found in Ripley (1987).

Smoothing

The mixture model (8) is a weighted estimate with kernel $T(\cdot|k,m,M)$. The theory for kernel estimation says that this mixture approaches $p(\cdot)$ for increasing sample size n if the bandwidth decays to zero at an appropriate rate. For a Gaussian kernel, a standard h is

$$h = \frac{d}{n^{1/u}}$$

with d given by

$$d = \left(\frac{4}{1+2s}\right)^{1/u}$$

Silverman (1986) defines $u = s + 4$ while West (1993) uses $u = 1 + 4s$ instead. For one-dimensional θ values, the two bandwidths are identical. If $q > 1$ (and n is fixed), West's formula gives comparatively larger values. Notice that the kernel is defined as a function of the sample size n and the dimension s of θ.

If multimodality is suspected, this standard bandwidth tends to oversmooth and slightly smaller values, say rh ($0.5 < r < 1$) are favoured. West (1993) also shows that, for fixed n, the mixture (8) is always overdispersed, which is a desirable feature for an importance function. The constant variance V in the Student kernel is replaced by its Monte Carlo estimate obtained from the current values of Θ and Ω. Furthermore, a crude $g_0(\cdot)$ need only capture global features of $p(\cdot)$ and a small sample size n_0 will do. A large sample will be drawn after the refined importance function has been obtained from the adaptive procedure.

Diagnostics

After each recursion in the adaptive reconstruction of (8), different diagnostics can be used to check for improvement in the importance function: (*i*) changes in (Monte Carlo) estimated expectations, (*ii*) configuration of points in each dimension of the parameter space Θ, (*iii*) frequency distribution of weights Ω, and (*iv*) measurement of variability of weights.

I shall use a measure of variability of weights as the standard diagnostic tool. West (1993) suggests the entropy relative to uniformity, which he defines as

$$(9)\qquad \frac{-\sum_{j=1}^{n} \omega_j \log \omega_j}{\log n}$$

This value is non-negative and equals unity in the case of a perfect match between $g(\theta_j)$ and $p(\theta_j)$ for all $\theta_j \in \Theta$; that is, the normalized weights ω_j will all equal $1/n$. As the variability among ω_j values increases, the relative entropy decreases. The choice of adequate sample size n_i, bandwidth h, and number of updates for $g(\cdot)$ can be guided by one or more of these diagnostics. For instance, look for stable Monte Carlo estimates of mean and variance (criteria 1); verify if the relative entropy (9) increases uniformly towards 1 (criteria 4), etc.

Reduction of mixtures

For a very large n, the mixture (8) might contain many redundancies, so that another mixture with a much smaller n is similar for all practical purposes (see West 1993 for examples). These smaller mixtures are obtained by reducing the n component sets $(\Theta^{(n)}, \Omega^{(n)})$ to the smaller pair $(\Theta^{(m)}, \Omega^{(m)})$ with $m < n$ (superscripts indicating cardinality of the sets), using the following clustering scheme.

(*i*) Sort the elements $\theta_j \in \Theta^{(n)}$ according to increasing weights $\omega_j \in \Omega^{(n)}$, such that θ_1 corresponds to the smallest weight ω_1.

(*ii*) Find the index i, such that θ_i is the nearest neighbour (i.e., Euclidean distance) to θ_1 and calculate

$$\omega^* = \omega_1 + \omega_i$$

$$\theta^* = \frac{\omega_1\theta_1 + \omega_i\theta_i}{\omega^*}$$

(*iii*) Drop θ_1 and θ_i from $\Theta^{(n)}$ and include θ^* instead, to get $\Theta^{(n-1)}$. Proceed similarly with ω_1, ω_i, and ω^* to get $\Omega^{(n-1)}$.

(*iv*) Repeat the previous steps ($n - m$) times, until obtaining $\Theta^{(m)}$ and $\Omega^{(m)}$.

The reduced mixture keeps the form (8) but has only m components in the sum, with locations $\Theta^{(m)}$ and weights $\Omega^{(m)}$. The estimate for V, needed in the kernel $T(\cdot|k,m,M)$ and calculated from the larger sets $\Theta^{(n)}$ and $\Omega^{(n)}$, is kept, but a larger bandwidth h, owing to the smaller number of kernels in the reduced mixture, is appropriate.

Sampling – importance – resampling algorithm

The SIR algorithm of Rubin (1988) consists of random draws of values θ^* from the set $\Theta = \{\theta_1, \ldots, \theta_n\}$ with the probability distribution given by $\Omega = \{\omega_1, \ldots, \omega_n\}$. This procedure is a weighted bootstrap that extends the equally likely resampling scheme of Efron (1982).

The resulting set $\Theta = \{\theta_1^*, \ldots, \theta_{n'}^*\}$ of size n' ($n' < n$) is approximately a sample of independent observations from $p(\cdot)$. This approximation becomes exact as $n/n' \to \infty$. I point out that the less $g(\cdot)$ resembles $p(\cdot)$, the larger n needs to be in order that the distribution of (the elements in) Θ^* well approximates $p(\cdot)$.

The SIR procedure is appealing in its simplicity and practicality. This results from the basic duality between sample θ^* and the probability density $p(\cdot)$ from which it is (approximately) generated. Given the sample, we can roughly recreate the density using graphical tools of exploratory data analysis (e.g., histograms, scatterplots) and summary statistics (e.g., mean, mode, quantiles, standard error). With these tools it is also easy to examine the distribution of functions $H(\theta)$ that are of interest.

Application: orange roughy

To illustrate the application of the AIS procedure to a biomass

dynamics model, I shall use data from Francis (1992) on the orange roughy (*Hoplostethus atlanticus*) fishery on the Chatham Rise, New Zealand. These data, reproduced in Table 1, include a time series of catches $\{C_t; t = 1, \ldots, 11\}$ and estimates of abundance from trawl surveys $\{I_t; t = 6, \ldots, 11\}$. Francis compared different policy options by assessing the risk of fishery collapses within the next 5 years. His risk analysis caused some recent controvery (Hilborn et al. 1993*b*; Walter 1993; Francis 1993). Here I perform a complete Bayesian decision analysis to reexamine the issue.

Table 1. Estimated catch history, and trawl survey biomass for orange roughy (*Hoplostethus atlanticus*) for the period 1978–1979 and 1988–1990.

Time	Catch (t)	Trawl index
1	15 340	—
2	40 430	—
3	36 660	—
4	32 354	—
5	20 064	—
6	32 263	164 835
7	38 142	149 425
8	39 098	102 975
9	39 896	80 397
10	31 478	97 108
11	42 621	66 291

Note: Data are from Francis (1992).

Model for biomass dynamics

I shall denote the biomass at time $t-1$ by B_{t-1} and describe its dynamic from $t-1$ to t by some function $G(\theta,B_{t-1})$ parameterized by $\theta \in S_\theta$. I also include the process noise x_t, a random variable to account for unexpected variation and model imperfection. Hence, I write the biomas dynamics as

$$B_t = G(\theta, B_{t-1})\, e^{x_t} \quad (10)$$

where $G(\theta,B_{t-1}) = S_{t-1} + aB_{t-1}(1 - bB_{t-1})$ for $t = 2, \ldots, 11$ and $S_t = B_t - C_t$, $b = 1/B_1$. As did Francis, I also assume negligible observation errors in C_t. Because biomass B_t is not observed directly, I need a model to relate this biomass to the observed abundance index I_t. My observation model is

$$I_t = F(\theta, B_t)e^{v_t} \quad (11)$$

where v_t is a random observation error and

$$F(\theta, B_t) = qB_t \text{ for } t = 6, \ldots, 11$$

The observation model (11) assumes direct proportionality between index I and the corresponding true biomass B, with constant of proportionality q.

The parameter θ is three dimensional and defined as

$$\theta = (\ln a, -\ln B_1, \ln q)'$$

These transformations of the original parameters a, B_1, and q range over the real line as required by the AIS procedure.

The multiplicative structure of the random components is a convenient choice because B_t and I_t are non-negative.

Francis built a cumulative distribution function for the virgin (unfished) biomass B_1 using an age-structured model with many parameters that were estimated elsewhere and are assumed known. This construction was done by replicating simulations 100 times and using a frequentist approach to make empirical estimates of cumulative probabilities. He proceeded by fitting a Johnson's distribution (Johnson and Kotz 1970, p. 23) to these data. Taking the first derivative of this fit, he obtained a probability density for B_1. Francis draws random values from this density to produce replicates for a frequentist construction of the risk function. The uncertainty about the virgin (unfished) biomass B_1 and how this uncertainty affects decision making is a key element in the controversy. Further uncertainties are ignored in the process.

I shall use the Bayesian approach and construct a joint posterior distribution for the parameter vector θ. This posterior distribution is further used in Bayesian decision making to determine an optimal action.

Determining priors

The first step in the analysis is the specification of a probability distribution to describe uncertainties about θ prior to the data. I used two different priors for θ. The first prior, denoted informative, assumes high confidence in prior identification of the mean values of the parameters. It is based on a guess for mean and a range for possible smallest and largest values. It represents my interpretation of the information provided and general assumptions used by Francis.

For the second prior, denoted diffuse, I used a different strategy. Starting with guesses for possible values of five prespecified quantiles (5, 25, 50, 75, and 95) I found the parameters of an idealized log-normal distribution that would best agree with those values. If these numbers were not coherent with such a distribution, I tried to improve with a new set of values. The parameters corresponding to the underlying Gaussian distribution were used as parameters in the Student prior. I also assumed a diagonal prior covariance.

For the parameter q I used as quantiles the values 0.20, 0.30, 0.41, 0.56, and 0.90; these values more than cover the range of possibilities considered by Francis. Similarly, for B_1 I used the values (in thousands) 200, 400, 650, 1000, and 2000 and for a the values 0.04, 0.10, 0.19, 0.49, and 0.95.

The prior parameters for the inverse gamma distribution, IG(α,β), of the observation variance V were the same in all runs. They were based on a coefficient of variation of 0.20 for observation errors in the abundance index, as was assumed by Francis. By fixing $\alpha_0 = 2$ and setting the mode for V at 0.40, the value $\beta_0 = 8.0$ was calculated.

The values for the prior were as follows: (*i*) $V \sim IG(\alpha_0 = 2.0, \beta_0 = 8.0)$, (*ii*) informative: $\pi_0(\theta) \sim T_3(9, m_0, C_0)$ with $m_0 = (-1{,}61, -13.36, -0.66)'$ and $C_0 = \text{diag}(0.30, 0.20, 0.05)$, and (*iii*) diffuse: $\pi_0(\theta) \sim T_3(9, m_0, C_0)$ with $m_0 = (-1{,}62, -13.37, -0.88)'$ and $C_0 = \text{diag}(0.97, 0.48, 0.21)$.

Likelihood function

For mathematical tractability of (11) it is convenient to take logarithms. Let $Y_t = \ln I_t$ and $\mu_t(\theta) = F(\theta,B_t)$. I use the observation model

$$p(Y_t|\theta, V) \sim N(\mu_t(\theta), V) \quad (12)$$

where $N(m,C)$ denotes a one-dimensional Gaussian distribution with mean m and variance C.

Prior uncertainties about θ and the nuisance parameter V are assumed to be independent and given by $\pi(\theta,V) = \pi(\theta)\pi(V)$.

For a given data set $Y(\tau) = \{Y_1, \ldots, Y_\tau\}$ I will be interested in features of the posterior distribution $p(\theta|Y(\tau))$, and sometimes in policy variables $H(\theta)$ or utilities $U(\theta,a)$. If $L(\theta,V) = p(Y(\tau)|\theta,V)$ is the likelihood for (θ,V) then, given the assumption of prior independence, I obtain $L(\theta) = p(Y(\tau)|\theta)$ by marginalization with respect to V. That is,

$$L(\theta) = \int p(Y(\tau)|\theta, \upsilon)\pi(\upsilon)d\upsilon$$

According to Bayes' theorem (1), the posterior for θ is obtained as

$$p(\theta|Y(\tau)) \propto L(\theta)\pi(\theta) \quad (13)$$

A key element of importance sampling is the evaluation of the ratio between the kernel $f(\theta)$ of the posterior (13) and the importance function $g(\theta)$ for any given value θ. The prior $\pi(\theta)$ is known by construction so that only the kernel of $L(\theta)$ needs to be found.

I shall use the solution proposed in Kinas (1993) (lemma 3.1, p. 50). There I assume an inverse-gamma prior $\pi(V) \sim IG(\alpha_0,\beta_0)$ and derive two special cases for the joint likelihood $L(\theta,V) = p(Y(\tau)|\theta,V)$. Assuming the observation model (12) and defining

$$Z(\theta) = \sum_{t=1}^{\tau} (Y_t - \mu_t(\theta))^2$$

the results are as follows:

(*i*) If $L(\theta,V) = \Pi_{t=1}^{\tau} p(Y_t|\theta, V)$, then

$$(14) \quad L(\theta) \propto \beta_\tau(\theta)^{\alpha_\tau}$$

where $\alpha_\tau = \alpha_0 + \tau/2$ and

$$\beta_\tau(\theta) = \frac{2\beta_0}{2 + \beta_0 Z(\theta)}$$

(*ii*) If $L(\theta, V) = \Pi_{t=1}^{\tau} p(Y_t|Y_{(t-1)}, \theta, V)$ and $(Y|Y_{(t-1)}, \theta) \sim lgn(\alpha_{t-1}, \beta_{t-1}(\theta))$, then

$$(15) \quad L(\theta) \propto \prod_{t=1}^{\tau} \frac{(\beta_t(\theta))^{\alpha_t}}{(\beta_{t-1}(\theta))^{\alpha_{t-1}}}$$

where $\alpha_t = \alpha_{t-1} + 1/2$

$$\beta_t(\theta) = \frac{2\beta_{t-1}(\theta)}{2 + \beta_{t-1}(\theta) Z_t(\theta)},$$

and $Z_t(\theta)$ is the t's term in the sum $Z(\theta)$.

In the first case the Y_t values are assumed to be independent while in the second case its sequential structure is taken into account by updating the parameters α and β for each time step t so that an up-to-date estimate of V is used for each data point Y_t. In all the cases I have analyzed, the benefits of using sequential learning of V were minor.

The model for biomass dynamics (10) is stochastic. Therefore, the values of $\mu_t(\theta)$ in (12) need to be estimated. For any fixed θ, I start by defining the time series of predicted biomasses:

$$(16) \quad \hat{B}_t = \begin{cases} E_\theta^w[B_t|\hat{B}_{t-1}] & \text{if } t \geq 2 \\ \hat{B}_1 \quad \text{fixed} \quad \text{possibly } \hat{B}_1 \in \theta \end{cases}$$

I further define the estimator $\hat{\mu}_t(\theta)$ by replacing B_t in (11) by its prediction $\hat{B}_t$. That is, $\hat{\mu}_t(\theta) = \ln F(\theta, \hat{B}_t)$. After drawing a sample of θ's from the prior $\pi(\theta)$, I propose the following procedure to calculate $f(\theta)$ for each fixed value θ. (*i*) Use the models (10) and (11) together with the definition (16) to obtain the sequence of predictions $\{\hat{\mu}_t(\theta); t = 1, \ldots, \tau\}$. (*ii*) Calculate $\hat{Z}_t = (Y_t - \hat{\mu}_t)^2$ for $t = 1, \ldots, \tau$. (*iii*) Use (14) or (15) together with the values $\{\hat{Z}_t(\theta); t = 1, \ldots, \tau\}$ to calculate the (estimated) likelihood $\hat{L}(\theta)$. (*iv*) Use $\hat{L}(\theta)$ instead of $L(\theta)$ when proceeding with AIS.

The procedure chosen to deal with the sequence of $\mu_t(\theta)$ emulates the observation error – time-series fitting procedure of Pella and Tomlinson (1969). Further justifications for definition (16) and discussions about $\hat{L}(\theta)$ are given in Kinas (1993).

Adaptive importance sampling calculations

I shall guide the reader through the various steps of the AIS algorithm for this specific example. A pseudocode for the procedure is available from the author on request.

- Step 1. Define the prior distribution $\pi(\theta)$. I used the three-dimensional Student distribution described above.
- Step 2. Define $g(\cdot) = \pi(\cdot)$. That is, use the prior distribution as the initial importance function.
- Step 3. Draw a sample of size $n_0 = 1000$ from $g_0(\cdot)$. This is the set

$$\Theta_0 = \{\theta_{0,j}; j = 1, \ldots, n_0\}$$

The sample size, an arbitrary choice based on the diagnostics and experimentation, is moderate. It is intended to capture crude features of the true posterior. In subsequent recursions the importance function becomes more refined. Therefore, the sample size will gradually increase.

- Step 4. For each $\theta_{0,j} \in \theta_0$ calculate: the likelihood $L(\theta_{0,j})$ using (14) or (15); the kernel of the posterior density $f(\theta_{0,j}) = L(\theta_{0,j})\pi(\theta_{0,j})$; the value $g_0(\theta_{0,j})$; the weight $\omega(\theta_{0,j}) = p(\theta_{0,j})/g_0(\theta_{0,j})$.
- Step 5. Calculate the set of normalized weights $\Omega_0 = \{w_{0,j}, j = 1, \ldots, n_0\}$ where $\omega_{0,j} = \omega(\theta_{0,j})/K$ and $K = \Sigma\, \omega(\theta_{0,j})$.
- Step 6. Using Θ_0 and Ω_0 calculate the Monte Carlo estimates of the (3×3) covariance matrix V, using (7). Also calculate the relative entropy for $g_0(\cdot)$ by using Ω_0 in equation (9).
- Step 7. Reduce the sets Θ_0 and Ω_0 to $m = 800$ points, using the procedure (reduction of mixtures) described in a previous section. Denote these new sets $\Theta_0^{(800)}$ and $\Omega_0^{(800)}$ and use them, together with the estimate V calculated in step 6, to construct the mixture model $g_1(\cdot)$ of the form (8). After fixing the kernel $d(\cdot|m,M)$ as a Student distribution with 9 degrees of freedom (to provide for heavy tails in the importance function), I experimented with various bandwidths h^2 between 0.6 and 1. For the results presented below, I used $h^2 = 0.64$.
- Step 8. Draw a new sample of size $n_1 = 2000$ from $g_1(\cdot)$ and denote it by $\Theta_1 = \{\theta_{1,j}, j = 1, \ldots, n_1\}$.
- Step 9. Repeat steps 4, 5, and 6 after replacing $\theta_{0,j}$ by $\theta_{1,j}$ and $g_0(\cdot)$ by $g_1(\cdot)$. Get the corresponding Ω_1, V, and the relative entropy for $g_1(\cdot)$.
- Step 10. Obtain the reduced sets $\Theta_1^{(800)}$ and $\Omega_1^{(800)}$. Construct the new mixture model $g_2(\cdot)$.
- Step 11. Repeat steps 8, 9, and 10 for $n_2 = 2500$ to obtain $g_3(\cdot)$. Repeat again the same steps generating $n_3 = n = 6000$ values $\theta_{3,j}$ from $g_3(\cdot)$. Keep the sets Θ_3 and Ω_3 and use them in the calculations of (6).
- Step 12. Obtain the reduced sets of $\Theta_3^{(800)}$ and $\Omega_3^{(800)}$. Use them in the final mixture model (8) to generate graphical representations of the posterior $p(\cdot)$.

Applying the sampling – importance – resampling method

The AIS procedure of the previous section is computer intensive. Also, the outcome (sets Θ and Ω) needs further treatment if a graphical display is intended because the relative importance of each value θ is given by the corresponding weight ω. In comparison, the SIR procedure is easy to handle, making it attractive and justifying its inclusion in the analysis.

The approximate SIR sample Θ^* becomes better when the importance function $g(\cdot)$ approximates the true posterior $p(\cdot)$ well. Therefore, it seems natural to resample from the final sets Θ and Ω obtained in AIS. Alternatively, one can generate the sets Θ and Ω from $\pi(\theta)$ directly, avoiding AIS completely. This is the approach used in standard applications of SIR (Smith and Gelfand 1992). Both situations are examined here.

From each of the previously specified priors (diffuse and informative), I generate a sample of $n = 10\,000$ points (Θ) and their standardized weights (Ω). I use these sets to resample $n' = 1000$ points to obtain Θ^*. Alternatively, starting from each prior, I first used AIS to get Θ and Ω. The resampling for θ^* was done as before. For the intermediate AIS steps I used small numbers (very workable on standard personal computers): $n_0 = 150$, $n_1 = 250$, $n_2 = 350$, and $m = 80$. Sample sizes $n_3 = n = 10\,000$ and $n' = 1000$ at the final stages were kept the same.

Results

Table 2 summarizes the results for the parameter of central interest: virgin biomass (B_1). Cases 1 and 2 refer to the AIS procedure while cases 3 to 6 use SIR and its association with AIS.

The relative entropy (9), a measure of closeness between the true posterior $p(\theta|\text{data})$ and the importance function $g(\theta)$,

Table 2. Posterior estimates of virgin biomass B_1 for orange roughy (values in 1000 t) and the probability (P) that B_1 exceeds 500 000 t for different stochastic simulation procedures and two priors: diffuse (D) and informative (I).

Case	Procedure	Prior	RE	B_1	P
1	AIS	D	0.98	431 (90)	0.18
2	AIS	I	0.99	384 (49)	0.02 (0.002)
3	SIR	D	0.70	447 (109)	0.20 (0.013)
4	AIS-SIR	D	0.97	437 (99)	0.20 (0.013)
5	SIR	I	0.69	396 (57)	0.05 (0.007)
6	AIS-SIR	I	0.98	389 (49)	0.02 (0.004)

Note: The relative entropy (RE) measures the closeness between the importance function and the true posterior (RE = 1 denotes a perfect fit).

follows the expected pattern: it increases towards 1, which denotes perfect fit at all the sample points. For example, case 2 in Table 2 gives the values 0.60, 0.95, 0.99, and 0.99 for the importance functions $g_i(\cdot)$ ($i = 0, \ldots, 3$, respectively; case 6 gives values 0.48, 0.83, 0.95, and 0.98.

To compare the three stochastic simulation procedures, I use this relative entropy (RE) and the posterior standard error of B_1 for each of the assumed prior distributions (Table 2). An improvement in the importance function is linked to a decrease in the posterior standard error of B_1 when moving from SIR to AIS-SIR and finally to AIS. For the diffuse prior (cases 3, 4, and 1) the pairs [RE, standard error] are [0.70, 1.09], [0.97, 0.99], and [0.98, 0.90], respectively. Changing to the informative prior (cases 5, 6, and 2), these values are [0.69, 0.57], [0.98, 0.49], and [0.99, 0.49].

The superiority of AIS-SIR over ordinary SIR is no surprise, because an improved importance function is used in the combined procedure. The interesting comparison is between AIS-SIR and AIS. While the combined AIS-SIR procedure is less computer intensive than the AIS alone, both procedures yield roughly the same results. This might become an important consideration in more complex models (or for Bayesian robustness analysis) where repeated application of AIS is very time consuming and clumsy. One could use the pure AIS procedure to produce a one-time pair of sets Θ and Ω and proceed by resampling repeatedly from these (Smith 1991).

The prior distribution $\pi(\theta)$ clearly influences the final estimates. If diffuse, the Monte Carlo estimates of posterior mean and standard error are higher (approximate averages 438 000 and 99 000 t) than estimates obtained under the informative alternative (390 000 and 52 000 t). Similarly, the estimate of the probability that B_1 exceeds 500 000 t (last column) is about 20% for the diffuse prior whereas it is at most 5% for the informative case.

The strong influence of different priors on the marginal posterior distribution of the virgin biomass B_1 is also displayed in graphics. The posterior densities for cases 1 and 2 (Fig. 1) as well as in the box plots of the posterior SIR samples for cases 3 to 6 (Fig. 2) show that for the diffuse prior values of B_1 up to 800 000 t are possible. This is quite different from the informative case, where values of B_1 exceeding 600 000 t are unlikely.

A comparison with the estimated density reported by Francis (1992) (his Fig. 1B) suggests the density that was obtained for case 2 (solid line in Fig. 1). In other words, the acceptance of Francis' distribution corresponds to the acceptance of the informative prior in my model.

The marked superiority of AIS in estimating the tail

Fig. 1. Marginal posterior distributions for virgin biomass (in thousands of tonnes) for different priors: informative (solid line) and diffuse (broken line).

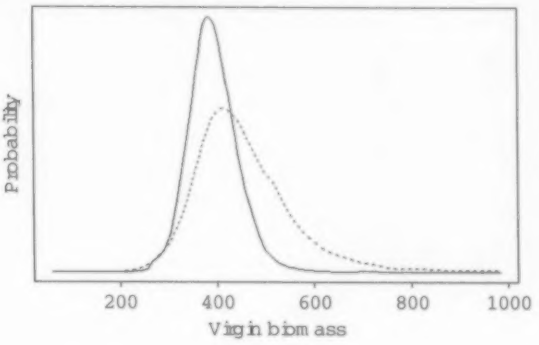


Fig. 2. Box plot of SIR posterior samples for virgin biomass (in thousands of tonnes). Cases 3 and 4 use a diffuse prior; cases 5 and 6 use an informative prior. In cases 3 and 5 the priors are also the importance functions whereas in cases 4 and 6 the adaptive generated mixture models (AIS) are used instead.

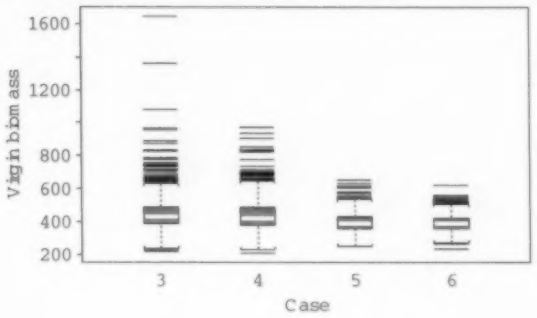


Fig. 3. Scatterplots of the SIR posterior sample for the two-dimensional marginal posterior distributions, using the informative prior and the AIS importance function (case 6).

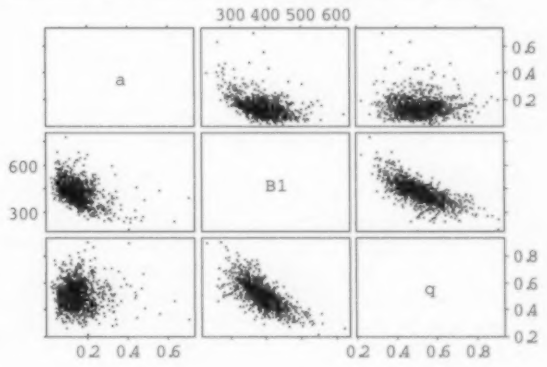
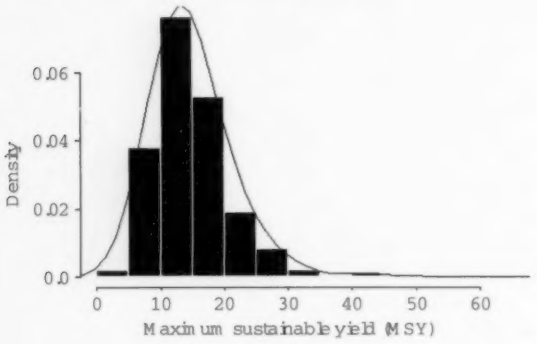


Fig. 4. Posterior probability density for maximum sustainable yield (MSY), given the informative prior and the AIS-generated importance function (case 6).



probability $P(B_1 > 500)$ is an effect of different sample sizes: 6000 points in cases 1 and 2, versus 1000 points in cases 3 to 6).

Other analyses and graphical displays are possible. For instance, the scatterplots of Fig. 3 show the posterior samples associated with the two-dimensional marginal distribution in case 6. Regions of high density can be identified. The asymmetric shape of the underlying distributions and the negative correlation between B_1 and q give a rough picture of the underlying distributions.

The posterior distributions of functions of θ are readily available from the SIR sample. I illustrate this in Fig. 4 with the posterior distribution of the maximum sustainable yield (MSY) for case 6. MSY is a function of parameters a and B_1 and defined as $\text{MSY} = aB_1/4$.

Decision making

The analysis so far has focused on the description of some general features of marginal uni- and bi-dimensional posterior distributions and their relation to prior specification. However, the final goal of Francis' analysis was the use of his risk assessment to make management decisions. Hilborn et al. (1993*b*) denounced the absence of a coherent framework for decision making under uncertainty.

I shall choose one of Francis' five policy options using Bayesian decision analysis. That is, three-dimensional posterior distribution for θ is coupled with a utility function to calculate the expected utility (2) for each policy a, to pick the option that maximizes the posterior expected utility (Bayes' rule). First I shall describe what these management options are.

The fishery for orange roughy is managed by annually setting the total allowable catch (TAC). For instance, the fishing seaason 1989–1990 (the year immediately following the data sequence of Table 1) had its TAC fixed at 28 637 t. According to Francis, there is an agreement to gradually reduce the TAC, but two questions remain: first, to what level should the TAC be reduced, and second, at what rate should the reduction take place.

The target level was fixed at a maximum constant yield of 7500 t, accounting for the first question. Francis defined this value to be ⅔MSY, where MSY denotes the deterministic maximum sustainable yield.

What I will try to answer here is the second question. That

Table 3. Risk-averse utilities ϕ associate to the five possible cumulative discounted catches ($CC^{(i)}$).

i	a_i	$CC^{(i)} \times 1^{0-3}$	$\phi(CC^{(i)})$
1	3	83	0.981
2	5	63	0.950
3	7	52	0.916
4	9	46	0.888
5	12	40	0.850

is, I will try to provide the rate for the reduction in TAC. Francis' Table 5 displays five alternative options. I will use the same options to define my set of actions $A = \{a_1, a_2, a_3, a_4, a_5\}$. Given action a_1, the rate of TAC reduction is 3000 t/year. Similarly for a_2 up to a_5, the rates are 5000, 7000, 9000, and 12 000 t/year, respectively.

Francis considers the risk to the fisheries of each option by estimating (using a frequentist empirical estimate) "the risk that the fishery would collapse within a five-year period." The fishery is said to have collapsed if, within this period, in some year t the TAC is greater than two-thirds of the biomass B_t.

To construct my utility function I assume that, for a fixed a_i and in any given year, the catch will equal the prescribed TAC; I denote this total quota by $T^{(i)}$. Hence, starting from the current $T_{(0)} = 28\,637$, future quotas for $s = 1, \ldots, 5$ are given by

$$T_s^{(i)} = \max\{T_{(0)} - sa_i; 7500\}$$

Defining the discount rate of future catches by δ, the cumulative discounted catch over the 5-year period is denoted $CC^{(i)}$ and given by

$$CC^{(i)} = \sum_{s=1}^{5} \delta^s T_s^{(i)}$$

I further need to identify the "occurrence of collapse given decision a_i." This is done by the indicator function

$$(17)\quad A^{(i)} = \begin{cases} 1 & \text{if } T_s^{(i)} > \frac{2}{3} B_{12+s} \text{ for some } s = 0, \ldots, 5 \\ 0 & \text{otherwise} \end{cases}$$

Given a pair $(\theta_j, a_i) \in \Theta \times A$ (recall that Θ is the random sample from the posterior distribution), the utility function can be stated in terms of the notation just introduced:

$$(18)\quad U(\theta_j, a_i) = (1 - A^{(i)})\phi(CC^{(i)})$$

where ϕ is some known nondecreasing function.

The utility (18) is zero whenever collapse is predicted ($A^{(i)} = 1$). Otherwise, it is $\phi(CC^{(i)})$. The dependence on the posterior $p(\theta|\text{data})$ becomes clear when considering the algorithm used to calculate (18):

- Step 1. Fix $\theta_j \in \Theta$ and use $G(\cdot)$ together with the observed catch data $\{C_t; t = 1, \ldots, 11\}$ to predict the average time series for $\{B_t; t = 2, \ldots, 12\}$.
- Step 2. Fix a decision rule $a_i \in A$ and define $\{T_0^{(i)}, T_1^{(i)}, \ldots, T_5^{(i)}\}$ to be the catches into the future.
- Step 3. Evaluate $A^{(i)}$ acording to (17).
- Step 4. Calculate $U(\theta_j, a_i)$ using (18).
- Step 5. Repeat the whole procedure for the next pair (θ_j, a_i).

I consider three alternatives for ϕ: (*i*) $\phi(x) = x$, (*ii*) $\phi(x) = 1$, and (*iii*) $\phi(x)$ is a risk-averse function defined in Table 3. The first assumes $\phi(x) = x$, so that the utility is linear in CC whenever the possibility for collapse is excluded. A more conservative approach, which is concerned only with the risk of collapse, defines $\phi(x) = 1$. This second alternative assumes the same utility for different catches regardless of size, an unrealistic assumption. A compromise between these two extremes can be obtained by a concave function for ϕ and is given as the third option. Such a function is said to be risk averse because it models the attitude of most people to avoid participating in a gamble where R dollars are gained or lost according to the outcome (heads or tail) of one throw of a fair coin. Although it is a fair game (the expected gain/loss ratio is zero), the prospect of losing R weighs more than the prospect of winning R (risk aversion).

Table 3 requires a few comments. Because the values $CC^{(i)}$ are fixed for each i, I only need to define ϕ for those particular points. Notice that the utilities become smaller with smaller values of CC, but the reduction is nonlinear. These values were determined assuming $\phi(160) = 1$ and using Table A.1 for constant risk aversion in Lindley (1985) (p. 196). For comparison, the corresponding linear (risk neutral) values would be 0.52, 0.39, 0.33, 0.29, and 0.25 for $i = 1$ to 5, respectively.

For the manager who seeks a practical answer, the results are in Table 4. Notice that the optimal rate depends on the utility function used. In Kinas (1993) I show that, for a fixed utility function, these decisions are robust over different prior assumptions.

If the linear relation $\phi(x) = x$ is chosen, the optimal decision is a_1, which recommends a reduction in TAC of 3000 t/year over the next 5 years. On the opposite end the conservative 0–1 option ($\phi(x) = 1$) recommends a_5, a drastic rate of annual reduction by 12 000 t. The intermediate risk-averse utility recommends rates in between: it chooses a_3, a reduction rate of 7000 t/year, as optimal.

The expected utilities in the 0–1 case have a special interpretation: the complement of the probability of collapse. For any decision a_i, the probability of collapse p_i is estimated as $p_i = 1 - E[U(\theta,a_i)]$. For instance, case 2 in Table 4 has estimates 0.27, 0.13, 0.08, 0.06, and 0.05 for actions a_1 to a_5, respectively (Kinas (1993) shows that these probabilities change little for a diffuse prior). These values disagree with the results presented in Francis' (1992) Fig. 3: his estimates of the risk of collapse exceed 0.6 for a_1, while probabilities smaller than 0.2 are observed for a_4 and a_5. As the two methods are derived under different paradigms, a direct comparison is not possible. However, Francis' model is complex (many parameters) and the estimates of risk are sensitive to recruitment assumptions (as indicated in his Fig. 3*d*). My model is comparatively simple and does not need special assumptions for recruitment. The findings in Ludwig and Walters (1985, 1989) indicate that simple models are likely to be more robust for management purposes.

Table 4. Expected utilities obtained for all five decision rules, according to the form of the utility function (expressed by ϕ).

ϕ	Case	a_1	a_2	a_3	a_4	a_5
Linear	1	0.622*	0.538	0.462	0.420	0.373
Risk averse	1	0.733	0.811	0.824*	0.817	0.796
	2	0.716	0.820	0.837*	0.828	0.810
0–1	2	0.730	0.870	0.918	0.934	0.951*

*The best decision.

Summary

There are three conclusions from this case study for orange roughy. Firstly, Francis (1992) computes only the risk of collapse associated with different actions. This is not sufficient for decision making. The Bayesian paradigm says that the maximization of expected utilities is the correct criteria to select one of these actions. I performed such an analysis with three distinct utility functions. By taking this additional step, my analysis completes the work of Francis.

Secondly, the shape of the marginal posterior distribution for B_1 is affected by the choice of prior. I used two alternatives: informative and diffuse. The distribution used by Francis corresponds to the informative prior. The diffuse prior resulted in a heavier upper tail, increasing the probability that $B_1 >$ 500 000 t from 2% to about 20%.

Thirdly, decision making is robust with respect to choice of prior. Given any of the utility functions, the optimal decision was not affected by the particular choice of prior (informative or diffuse). This indicates that for the current model or data situation, and assuming consensus about the set of actions A, the tail behaviour in the distribution for B_1 was not strong enough to change the overall optimal decision.

Discussion

Some features of the proposed Bayesian procedure need further comments. The observation error variance V and the system noise W are seen as nuisance variables and are therefore eliminated from the problem. Variance V is eliminated by marginalization. To use the conjugate property of distribution, I use an informative inverse-gamma distribution as a prior. Another possibility is to use Jeffrey's (1961) noninformative prior $\pi(V) \propto 1/V$. If I further chose the independence assumption for the observations Y_t, the likelihood is given by the following equation (see Appendix for the derivation):

$$(19) \quad L(\theta) \propto Z(\theta)^{\frac{-(n-1)}{2}}$$

This simplifies calculations and avoids the need to specify the parameters α_0 and B_0 for the inverse-gamma prior. It is an alternative when no information about V is available or the inverse-gamma prior is to be avoided.

To eliminate W I borrow the idea of Pella and Tomlinson's (1969) observation error – time-series fitting. A similar rationale is used in the Bayesian synthesis approach (Givens et al. 1993). I replace the stochastic sequence $\{B_t\}$ by the deterministic alternative $\{\hat{B}_t\}$ defined in (16). A better definition would be in the terms of μ_t values. That is,

$$\hat{\mu}_t(\theta) = E[\mu_t(\theta)|\hat{\mu}_{t-1}(\theta)]$$

For the observation model (11) this implies use of the expectation of log B_t instead.

For the orange roughy case study I consider one particular surplus-production model. Kinas (1993) further applies the AIS procedure to models for stock recruitment and delay difference. In fact, the method is much more general because likelihood functions (14), (15), and (19) only require the

evaluation of the squared differences $Z_t(\theta) = (Y_t - \mu_t(\theta))^2$ for $t = 1, \ldots, \tau$. Any model capable of producing estimates $\hat{\mu}_t(\theta)$ can be used.

Parameter estimates in the form of posterior probability distributions provide a wealth of information. There is, for instance, the potential to detect multimodality. This feature indicates that more than one model is supported by the data. Identification of alternative hypotheses and the corresponding probabilities is an important tool in the design of adaptive management policies (Walters 1986). However, the implications of multimodality on management strategies and the benefits gained from a Bayesian analysis, as compared with the traditional approach based on a single best model, need to be investigated more extensively.

The goal of this paper was to develop tools for the practical implementation of Bayesian statistical analysis in fisheries management. A stochastic simulation procedure combining the virtues of a good importance function with the simplicity of the sampling – importance – resampling algorithm was proposed. Despite the encouraging results, the proposed procedure needs to be tested more carefully. In particular, a study that compares computer efficiency and the precision of the estimates with those produced by alternative methods is necessary.

The goal of this paper was to develop tools for the practical implementation of Bayesian statistical analysis in fisheries management. A stochastic simulation procedure combining the virtues of a good importance function with the simplicity of the sampling – importance – resampling algorithm was proposed. Despite the encouraging results, the proposed procedure needs to be tested more carefully. In particular, a study that compares computer efficiency and the precision of the estimates with those produced by alternative methods is necessary.

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References

Abramowich, M., and Stegun, I.A. 1964. Handbook of mathematical functions. National Bureau of Standards, Washington, D.C.

Berger, O.J. 1985. Statistical decision theory and bayesian analysis. Springer Verlag, New York.

Efron, B. 1982. The jacknife, the bootstrap and other resampling plans. SIAM-CBMS, **38**: 1–92.

Francis, R.I.C.C. 1993. The interpretation of "probability": a response to Walters comment. Can. J. Fish. Aquat. Sci. **50**: 882–883.

Geweke, J. 1989. Bayesian inference in econometric models using Monte Carlo integration. Econometrica, **57**: 1377–1339.

Givens, G.H., Raftery, A.E., and Zeh, J.E. 1993. Benefits of a Bayesian approach for synthesizing multiple sources of evidence and uncertainty linked by a deterministic model. Rep. Int. Whal. Comm. **43**: 495–500.

Givens, G.H., Raftery, A.E., and Zeh, J.E. 1994. A reweighting approach for sensitivity analysis within the Bayesian synthesis framework for population assessment modeling. Rep. Int. Whal. Comm. **44**: 377–383.

Hilborn, R., Pikitch, E.K., and Francis, R.C. 1993*a*. Current trends in including risk and uncertainty in stock assessment and harvest decisions. Can. J. Fish. Aquat. Sci. **50**: 874-880.

Hilborn, R., Pikitch, E.K., McAllister, M.M., and Punt, A. 1993*b*. A comment on "Use of risk analysis to assess fishery management strategies: a case study using orange roughy (*Hoplostethus atlanticus*) on the Chatham Rise, New Zealand" by R.I.C.C. Francis. Can. J. Fish. Aquat. Sci. **50**: 1122–1125.

Jeffreys, H. 1961. Theory of probability. 3rd ed. Oxford University Press, London.

Johnson, N.L., and Kotz, S. 1970. Continuous univariate distributions. Houghton Mifflin, Boston.

Kinas, P.G. 1993. Bayesian statistics for fishery stock assessment and management. Ph.D. thesis, Department of Statistics, University of British Columbia, Vancouver, B.C.

Kloek, T., and van Dijk, H.K. 1978. Bayesian estimates of equation system parameters: an application of integration by Monte Carlo. Econometrica, **46**: 1–19.

Ludwig, D., and Walters, C.J. 1985. Are age structured models appropriate for catch-effort data? Can. J. Fish. Aquat. Sci. **42**: 1066–1072.

Ludwig, D., and Walters, C.J. 1989. A robust method for parameter estimation from catch and effort data. Can. J. Fish. Aquat. Sci. **46**: 137–144.

Naylor, J.C., and Smith, A.F.M. 1988. Econometric illustrations of novel numerical integration strategies for Bayesian inference. J. Econometrics, **38**: 103–125.

Pella, J.J., and Tomlinson, P.K. 1969. A generalized stock-production model. Bull. Int.-Am. Trop. Tuna Comm. **13**: 419–496.

Ripley, B. 1987. Stochastic simulation. John Wiley & Sons, New York.

Rubin, D.B. 1988. Using the SIR algorithm to simulate posterior distributions. *In* Bayesian Statistics 3: Proceedings of the Third Valencia International Meeting, Valencia, Spain. *Edited by* J.M. Bernardo, M.H. DeGroot, D.V. Lindley, and A.F.M. Smith. Clarendon Press, Oxford.

Silverman, B.W. 1986. Density estimation for statistics and data analysis. Chapman and Hall, London.

Smith, A.F.M. 1991. Bayesian computational methods. Philos. Trans. R. Soc. Lond. A Math. Phys. Sci. **337**: 369–386.

Smith, A.F.M., and Gelfand, A.E. 1992. Bayesian statistics without tears: a sampling–resampling perspective. Am. Stat. **46**: 84–88.

Walters, C.J. 1986. Adaptive management of renewable resources. McMillan Publishing Co., New York.

Walters, C.J. 1993. Comment on R.I.C.C. Francis: computing probability distributions for risk analysis. Can. J. Fish. Aquat. Sci. **50**: 881–882.

West, M. 1992. Modelling with mixtures. *In* Bayesian Statistics 4: Proceedings of the Fourth Valencia International Meeting, Valencia, Spain. *Edited by* J.M. Bernardo, J.O. Berger, A.P. Dawid, and A.F.M. Smith. Clarendon Press, Oxford.

West, M. 1993. Approximating posterior distributions by mixtures. J. R. Stat. Soc. B, **55**: 409–422.

Wolpert, R.L. 1991. Monte Carlo integration in Bayesian statistical analysis. Contemp. Math. **115**: 101–116.

Appendix

Form of $L(\theta)$ given Jeffreys' prior

To derive (19), I begin with the definition of the gamma function (Abramovitch and Stegun 1964, p. 255):

$$(20)\quad \Gamma(p) = \int_0^\infty u^{p-1} \exp(-u)du$$

Setting $p = -m$ and making the change of variables $u = -A/v$ the following relation is derived:

$$(21)\quad \int_0^\infty v^{-(m+1)} \exp(-A/v)dv = \frac{\Gamma(m)}{A^m}$$

Using the prior $\pi(v) \propto 1/v$, the likelihood $L(\theta)$ has the form

$$(22)\quad L(\theta) \propto \int_0^\infty v^{-\left(\frac{n+1}{2}\right)} \exp\left(-\frac{Z(\theta)}{2v}\right)dv$$

However, the integral in (22) is equivalent to (21), with $m = (n-1)/2$ and $A = Z(\theta)/2$. Retaining only the term in θ results in (19).

Mitochondrial DNA diversity in anadromous rainbow smelt, *Osmerus mordax* Mitchill: a genetic assessment of the member–vagrant hypothesis

Louis Bernatchez and Sylvain Martin

Abstract: We analyzed mitochondrial DNA genetic variance in anadromous smelt to test whether the ecological processes inferred by the member–vagrant hypothesis to explain spatial patterns of abundance may also have evolutionary consequences for the process of population divergence. Restriction enzyme analysis was performed on 568 adult smelt from 16 sites within the estuary and the Gulf of St. Lawrence (Canada). Hierarchical analysis of heterogeneity in the frequency of 140 mitochondrial DNA genotypes and clustering analysis revealed the existence of four distinct populations among which gene flow has been restricted. This population structure appeared to be organized by the number of suitable habitats for retention of early life-history stages and their probability of diffusion among populations. This suggests that ecological processes inferred by the member–vagrant hypothesis may be a primary factor controlling population genetic diversity in anadromous smelt. Biogeographic events may also have played a major role in shaping smelt population genetic diversity. Our results emphasize the importance of considering the interplay between the life-history characteristics of a species, its genetic diversity, and environmental constraints in a historical perspective to better understand its evolutionary ecology.

Résumé : Nous avons analysé la variance génétique de l'ADN mitochondrial chez l'Éperlan arc-en-ciel anadrome afin de vérifier si les processus écologiques expliquant les patrons de distribution spatiale selon l'hypothèse de « member–vagrant » pouvaient également avoir des conséquences évolutives sur le processus de divergence des populations. Un total de 568 éperlans adultes provenant de 16 sites de l'estuaire et du golfe du Saint-Laurent (Canada) ont été analysés par enzymes de restriction. Les analyses de groupement et hiérarchique de l'hétérogénéité de la fréquence de 140 haplotypes mitochondriaux ont mis en évidence quatre populations distinctes entre lesquelles le flux génique est restreint. Cette structure populationnelle semble correspondre au nombre d'habitats favorables à la rétention des jeunes stades et à leur probabilité de diffusion entre populations. Ces résultats suggèrent que les processus écologiques sous-jacents à l'hypothèse de « member–vagrant » ont un effet déterminant sur la diversité génétique de l'Éperlan. De plus, des facteurs biogéographiques ont probablement joué un rôle important sur la détermination de cette diversité. Dans l'ensemble, cette étude illustre l'importance de considérer dans une perspective historique les interactions entre les caractéristiques du cycle vital d'une espèce, sa diversité génétique et les contraintes environnementales afin de mieux en comprendre l'écologie évolutive.

Introduction

Explaining the extent, causes, and consequences of biotic distribution in space is fundamental to our understanding of how species evolve to cope in particular environments. In the past decade, the member–vagrant hypothesis has provided a major theoretical framework for studying the role of ecological processes in determining spatial patterns of abundance in aquatic species, particularly for marine ecosystems (Iles and Sinclair 1982; Sinclair 1988; Sinclair and Iles 1988, 1989). This hypothesis proposes that the population structure of a species evolves primarily as a consequence of selective forces that maximize the probability of encounter among sexually mature individuals and the survival of early life-history stages. One prediction of this hypothesis is that the number of populations composing a given species will primarily be determined by the number of environmental settings (geographic, physical, oceanographic) to which (*i*) spawning adults are adapted to home and (*ii*) early life history is behaviorly adapted to permit retention. This implies that in situations where the distribution of larvae is different from the distribution of spawning sites, population structure should reflect the number of retention zones rather than that of spawning sites (Sinclair 1988).

The member–vagrant hypothesis has been supported by evidence of a correlation between the number of early life-history retention zones and the number of distinct fish groups determined almost exclusively by geographic discreteness, phenotypic variation in life-history traits, or other biological characteristics that may reflect locally varying environmental factors (reviewed in Sinclair 1988). In contrast, little attention has been given to the impact that such processes operating at the ecological scale could have at an evolutionary scale, namely by restricting gene flow among groups associated with different retention zones, thus enhancing potential for local adaptation and ultimately promoting reproductive isolation.

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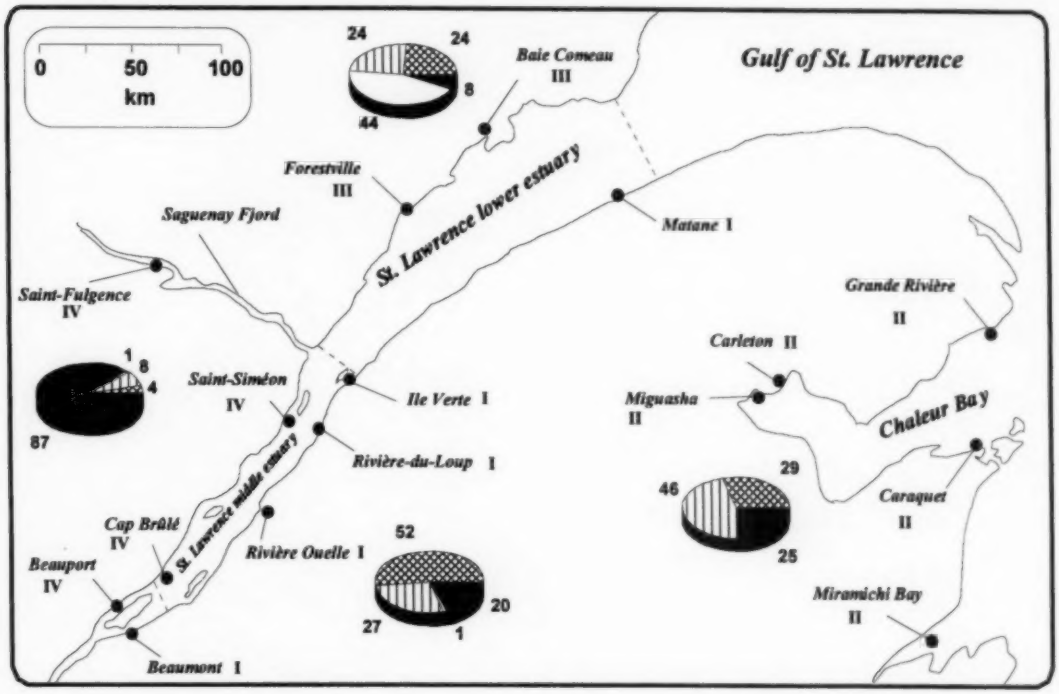
Fig. 1. Collection sites and frequency distribution of mtDNA haplotypes among anadromous smelt from the estuary and Gulf of St. Lawrence. Frequencies are given as percentages for pooled sampling sites of each of the four populations (I to IV) defined in Fig. 3: all mtDNA group B genotypes (solid), subcluster A11 (open), single haplotype 1 (hatched), and all other mtDNA group A genotypes (vertical lines).



The hypothesis that these ecological processes may have evolutionary consequences for population divergence would be supported by a correlation between the number of groups associated with potential retention zones and significant partitioning of genetic variance among them. Available genetic data do not clearly support this prediction. On one hand, the genetic population structure of species found at both extremes of the "continuum of population richness" (Fig. 5.12 of Sinclair 1988), such as American eel, *Anguilla rostrata* (Avise et al. 1986), and Atlantic menhaden, *Brevoortia tyrannus* (Bowen and Avise 1990), which are population poor, or Atlantic salmon, *Salmo salar* (reviewed in Davidson et al. 1989), American shad, *Alosa sapidissima* (Bentzen et al. 1989), and striped bass, *Morone saxatilis* (Chapman 1990; Wirgin et al. 1993), which are population rich, conforms to the species' number of retention zones. In contrast, null or very low genetic subdivision has generally been reported in species characterized by an intermediate number of retention zones, such as Atlantic cod, *Gadus morhua* (Carr and Marshall 1991), haddock, *Melanogrammus aeglefinus* (Zwanenburg et al. 1992), Atlantic herring, *Clupea harengus harengus* (Kornfield and Bogdanowicz 1987), capelin, *Mallotus villosus* (Dodson et al. 1991), and American plaice, *Hippoglossoides platessoides* (Stott et al. 1992). While these results may truly reflect genetic homogeneity among groups associated with distinct retention zones, it is also possible that in some cases, the sample sizes or specific levels of polymorphism for the genetic markers used were inappropriate to obtain statistically significant results (Ovenden 1990; Ferguson 1994).

Rainbow smelt, *Osmerus mordax,* is native to watersheds tributary to the western Atlantic Ocean from New Jersey to the Labrador coast (Scott and Crossman 1973). Anadromous populations of this species exhibit a similar life cycle: adult smelt ascend streams and rivers in early spring and spawn in fresh water. Hatching occurs 1–2 weeks later and larvae from different spawning sites are usually transported within short periods into estuarine or marine coastal waters where they are retained in estuaries (e.g., Ouellet and Dodson 1985; Laprise and Dodson 1989*a*, 1989*b*), fjords, or coastal embayments until metamorphosis. In Quebec waters, previous studies of variation in biological characters and parasite occurrence supported the proposition that the number of phenotypically distinct smelt groups reflects the number of larval retention zones rather than the number of spawning sites (Fréchet et al. 1983*a*, 1983*b*). These studies revealed the existence of three phenotypic smelt groups: the south shore of the St. Lawrence middle estuary, Chaleur Bay, and the Saguenay Fjord (Fig. 1). In addition, the existence of a fourth group along the north shore of the lower estuary was suggested.

In recent years, the analysis of mitochondrial DNA (mtDNA) variation has proven most useful in assessing the genetic structure of smelt populations (Baby et al. 1991; Taylor and Bentzen 1993*a*, 1993*b*; Taylor and Dodson 1994). Namely, a restriction analysis of mtDNA variation supported

Table 1. Location, collection date, sample size, number of mitochondrial genotypes, and nucleon diversity (h) estimates for 16 anadromous smelt samples from the estuary and Gulf of St. Lawrence.

Sampling site	Region	Date	n	No. of genotypes	h
Beaumont	I	April 1990	36	13	0.73
Rivière-Ouelle	I	May 1990	36	14	0.75
Rivière-du-Loup	I	Nov. 1992	36	14	0.69
Île Verte	I	Dec. 1992	36	12	0.71
Matane	I	Nov. 1992	36	13	0.73
Grande-Rivière	II	Oct. 1993	36	19	0.83
Rivière Nouvelle	II	May 1990	36	24	0.95
Miguasha	II	Jan. 1994	36	24	0.91
Caraquet	II	Oct. 1993	36	17	0.87
Miramichi River	II	May 1991	36	23	0.95
Forestville	III	Jan. 1993	36	15	0.83
Baie Comeau	III	Jan. 1993	35	11	0.78
Beauport*	IV	May 1993	36	—	—
Cap-Brûlé	IV	Oct. 1992	30	12	0.74
Saint-Siméon	IV	Nov. 1992	33	13	0.87
Saint-Fulgence	IV	March 1993	35	11	0.71

Note: Region refers to the geographic population designation as defined in Figs. 1 and 3.

*The number of genotypes and nucleon diversity were not quantified for Beauport as individuals of that site were successfully characterized for only a subset of restriction enzymes allowing us to classify them to major phylogenetic groups A and B (Fig. 2).

the hypothesis that smelt from the south shore of the St. Lawrence middle estuary composed a single population, genetically distinct from smelt of Chaleur Bay (Baby et al. 1991). The limited number of samples, however, precluded the partitioning of genetic variance among the four regional smelt groups proposed by Fréchet et al. (1983*a*, 1983*b*).

In this study, we performed a more comprehensive restriction fragment length polymorphism (RFLP) mtDNA analysis of anadromous smelt to test the hypothesis that ecological processes inferred by the member–vagrant hypothesis to explain patterns of geographic and phenotypic discreteness in aquatic species also translate into significant genetic subdivision, and therefore, may have evolutionary consequences for the process of population divergence.

Material and methods

Sample collection

An average of 36 reproductive females were obtained between 1990 and 1994 from 16 sampling sites covering the distribution of the four smelt groups described by Fréchet et al. (1983*a*, 1983*b*) as well as the Miramichi River, New Brunswick (Fig. 1, Table 1). Fish were captured either on spawning grounds or in nearby wintering areas, with the exception of one sample (Saint-Siméon) that was obtained from a fall commercial fishery. The four samples obtained in 1990–1991 were those previously analyzed by Baby et al. (1991). mtDNA was extracted from fresh or frozen ovaries according to Chapman and Powers (1984) as modified by Bernatchez et al. (1988). Restriction enzymes, electrophoresis, and digests procedures were the same as in Baby et al. (1991). This methodology insured complete compatibility with data obtained in that study. Six hexameric (*Apa*I, *Bgl*I, *Bst*EII, *Dra*I, *Eco*RV, and *Pst*I), four multihexameric (*Ava*I, *Ban*I, *Ban*II, and *Hinc*II), and two multipentameric (*Ava*II, *Nci*I) restriction enzymes were used.

Data analysis

Composite mtDNA genotypes were defined by distinct combinations of polymorphic restriction sites observed across all restriction enzymes. Relationships among genotypes were assessed by using the restriction site presence–absence matrix to generate phylogenetic trees according to Wagner parsimony criteria using the MIX program of the PHYLIP 3.5c computer package (Felsenstein 1993). A majority-rule consensus tree was constructed using the CONSENSE program and confidence statements on branches were estimated by running the MIX program on 100 bootstrapped matrices of the restriction site data generated by the SEQBOOT program.

Relationships among all samples were estimated by computing Nei's (1978) genetic distance (D), treating mtDNA genotypes as alleles of a single locus. The resulting pairwise matrix of genetic distance estimates was used to construct a phenogram relating samples using the unweighted pair-group method of arithmetic averages (UPGMA, Sneath and Sokal 1973). The significance of population clustering was assessed by a hierarchical analysis of heterogeneity in the frequencies of mtDNA genotypes (Sokal and Rohlf 1981), using χ^2 randomization tests (Roff and Bentzen 1989) with 1000 permutations performed by the MONTE program of the REAP software package (McElroy et al. 1992). The heterogeneity test was first performed on the overall population set, and then among population clusters, beginning with the most divergent. This χ^2 decomposition procedure was performed until no significant heterogeneity was detected.

The geographic differentiation of mtDNA variation was further assessed using the analysis of molecular variance model (AMOVA) of Excoffier et al. (1992). This procedure calculated standard variance components, and an array of haplotypic correlation measures, referred to as Phi statistics for different levels of population. The significance of the observed values for those parameters was tested using a random permutation procedure available in the AMOVA computer package. This avoids the parametric assumptions of normality and independence that are rarely met by genetic distance measures. By this approach, we computed the variance components resulting from molecular differences at three hierarchical levels: among groups, among populations within groups, and within populations. The definition of groups was based on the number of significant clusters depicted in the population phenogram. We also computed pairwise fixation indices (F_{st}) among significant groups by omitting the molecular information of divergence among mtDNA genotypes and simply considering those as multiple alleles of a single locus (Excoffier et al. 1992). This also provided an estimate of the effective number of females exchanged per generation among groups according to the approximation $N_e m_f = (1/F_{st}-1)/2$. Although the veracity of the absolute $N_e m_f$ estimates depends on several assumptions that may not be met in the present situation ($m \ll 1$, population in equilibrium with respect to genetic drift and migration, selective neutrality, island model of population structure), they nevertheless provide a comparative basis for estimating differential gene flow among populations.

Results

mtDNA diversity and phylogenetic differentiation

The 12 restriction enzymes used allowed the recognition of 197 different restriction sites, including 68 synplesiomorphies, 84 autapomorphies, and 45 synapomorphies. These resolved a total of 140 mtDNA genotypes among 568 smelt that were screened, of which 58 were previously reported in Baby et al. (1991). A complete description of composite haplotypes and

Fig. 2. Unrooted majority-rule consensus network showing relationships among 140 mtDNA genotypes identified in anadromous smelt from the estuary and the Gulf of St. Lawrence. Bootstrap confidence statements higher than the 50% majority-rule criterion are given along branches. Numbers at the tips of branches identify genotypes with their total abundance given in parentheses. The scale of 0.001 substitution/site corresponds to one restriction site difference.

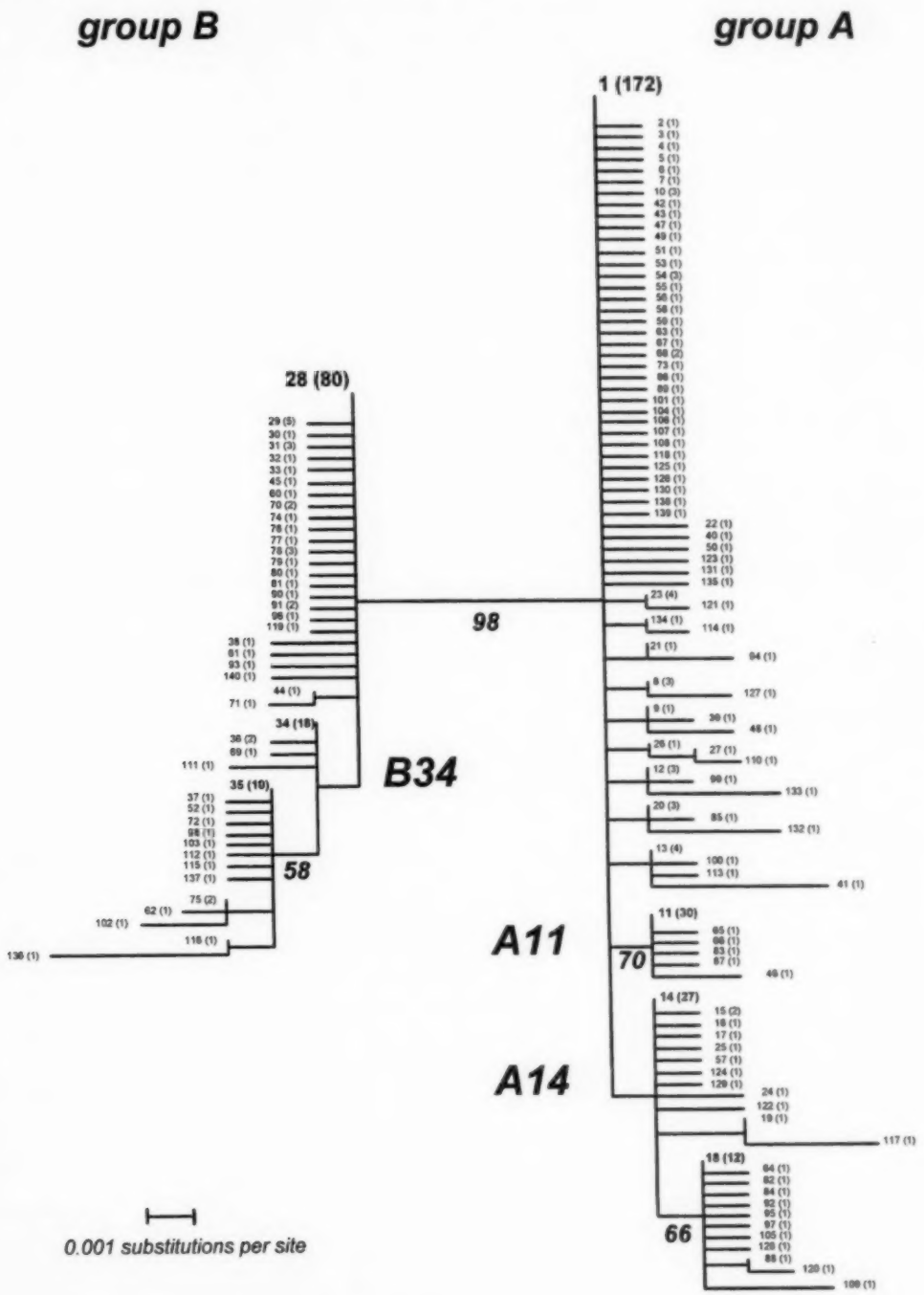


Fig. 3. UPGMA population tree clustering anadromous smelt samples into four population groups (I to IV) according to the matrix of Nei's genetic distance. Levels of significance (*P*) in the frequency distribution of mtDNA genotypes among clusters are given along branches. The clustering of the Beauport sample was only based on the relative frequency of mtDNA groups A and B as not all restriction enzymes were resolved for that site.

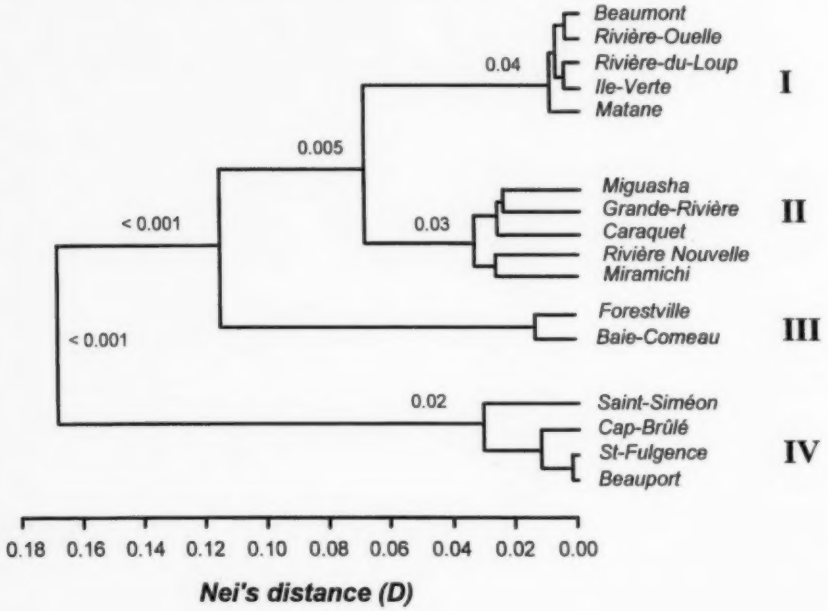


Table 2. The hierarchical analysis of molecular variance of mitochondrial DNA genotypes among anadromous smelt of the estuary and Gulf of St. Lawrence.

Variance component	df	% total variance	Phi statistic	*P*
Among groups	3	22.38	CT=0.224	0.001
Among samples within group	11	0.00	SC=0.000	0.672
Within samples	507	77.62	ST=0.221	0.001

their frequency distribution is provided elsewhere (Bernatchez 1995; Bernatchez et al. 1995).

As illustrated by the topology of the unrooted majority-rule consensus tree (Fig. 2), all mtDNA genotypes clustered into two monophyletic mtDNA groups (A and B) that were supported at a 98% bootstrapping level. These two groups were differentiated by six apomorphies generated by the restriction enzymes *Apa*I, *Ava*II ($n = 2$), *Ban*II, *Hinc*II, and *Nci*I, and the average pairwise sequence divergence among genotypes from both groups was 0.83%. Within each of them, the overall topology was a starlike phylogeny in which a central genotype (genotype 1 in group A, genotype 28 in group B) dominated in abundance and was surrounded by a majority of rarer genotypes generally derived by one or two mutational steps. Additional subclustering, however, was also observed. In phylogenetic group A, subclusters A11 and A14 were composed of 6 and 23 genotypes, respectively, sharing a unique apomorphy in each case. Subcluster A11 was supported at a 70% bootstrapping level whereas A14 was not significant under the 50% majority-rule criterion. Within group B, subcluster B34 comprised 17 genotypes sharing a unique apomorphy. However, only the branch grouping genotypes 35–137 obtained significant support under the 50% majority-rule consensus criterion.

Population diversity and relationships

The population UPGMA phenogram generated from Nei's genetic distance matrix clustered the 16 samples into significant clusters exhibiting a clear geographic pattern of distribution (Figs. 1, 3). Group I included all samples from the south shore of the St. Lawrence estuary, group II those from Chaleur Bay and Miramichi River, group III those from the north shore of the St. Lawrence lower estuary, and group IV those from the north shore of the St. Lawrence middle estuary and the Saguenay Fjord. Genetic distances among samples within each cluster were low and significant differences in haplotype frequencies were observed in only three comparisons. Within group II, the Nouvelle and Miramichi River samples differed slightly from the others, as did the Matane sample within group I and the Saint-Siméon sample within group IV.

The four groups were characterized by a distinctive mtDNA feature (Fig. 1). The smelt assemblage from the north shore of the St. Lawrence middle estuary and the Saguenay Fjord (group IV) was largely dominated (87%) by genotypes belonging to phylogenetic group B, which was much less abundant among other groups. Smelt samples from the north shore of the St. Lawrence lower estuary (group III) were characterized by a high frequency of genotypes (44%) belonging

Table 3. Pairwise comparisons of Phi-CT (below main diagonal) and F_{st} (above) coefficients among four genetically distinct anadromous smelt populations from the estuary and Gulf of St. Lawrence.

Population	I	II	III	IV
I. St. Lawrence middle–lower estuary (south shore)	0.000	0.032 (15.0)	0.132 (3.3)	0.178 (2.3)
II. Chaleur Bay – Miramichi River	0.008	0.000	0.075 (6.2)	0.100 (4.5)
III. St. Lawrence lower estuary (north shore)	0.087	0.089	0.000	0.181 (2.3)
IV. St. Lawrence middle estuary (north shore) – Saguenay Fjord	0.407	0.323	0.551	0.030

Note: Indirect estimates of long-term migratory exchange of females per generation (N_em_f) among populations are given in parentheses. The main diagonal gives the F_{st} coefficients estimated among sites within each population.

to subcluster A11, which was observed in only three individuals elsewhere. The St. Lawrence south shore and Chaleur Bay groups (I and II) were less distinctive; the former showed a higher proportion of the single genotype 1 whereas the latter was characterized by a high occurrence of rare genotypes belonging to phylogenetic group A. The high occurrence of rare genotypes in Chaleur Bay also translated into nucleon diversity (h) values that were higher (t value = 7.07, df = 8, $P < 0.0001$) than those observed for group I (Table 1).

Components of genetic variance

The analysis of molecular variance components also supported the patterns of high homogeneity within and significant genetic heterogeneity among the four smelt groups (Table 2). Thus, the nested analysis of population structure revealed that 22.38% of the total mtDNA genotypic diversity was explained by the variance among the four groups, whereas variance among sites within groups was null. Most of the overall diversity (77.62%) was attributable to variability within populations. The permutation procedure showed that the partitioning of variance among groups (Phi-CT) and within samples (Phi-ST) was highly significant.

Pairwise comparisons of molecular variance (Phi-CT estimates) among groups were highly variable. The lowest differentiation was observed between group I (St. Lawrence south shore) and group II (Chaleur Bay) for which molecular variance was less than 1% (Table 3). This suggested relatively high effective migration rates between the two regional populations, as reflected by the N_em_f value of 15.0. Intermediate values were observed between group III (north shore of the St. Lawrence lower estuary) and the latter two populations. Clearly, group IV was the most distinctive, with 32–55% of the total genetic diversity explained by intergroup variance when compared with any of the other regional populations, including that associated with the south shore of the St. Lawrence estuary, for which samples were generally obtained less than 20 km away.

Discussion

mtDNA diversity in anadromous smelt

To reveal significant population structure, a genetic technique must show levels of variation commensurate with the questions being addressed. The overall mtDNA genotypic diversity in anadromous smelt observed in this study ($h = 0.858$) was above that generally observed in other anadromous species (compiled in Billington and Hebert 1991). As reported in previous studies of mtDNA variation in smelt, haplotypes grouped into two phylogenetic groups (A and B), within which several individual genotypes or groups of genotypes were found at moderate to high frequencies, thus representing potentially informative markers to statistically address variation in genotypic frequencies and genetic diversity.

The high level of haplotype diversity coupled with the statistical analysis of genetic variance partitioning revealed a highly significant population structure in anadromous smelt. The 16 samples analyzed clustered into four groups showing a sharp geographic distribution. The genetic distinctiveness of these groups was supported by genetic distance values and significant differences in mtDNA haplotype distribution, as well as significant molecular variance estimates in pairwise comparisons among all groups. Within-group estimates of these parameters were generally null and not statistically significant, which suggests that extensive gene flow occurs among sites within each group. These results suggest that the anadromous smelt covered in this study comprise four genetically distinct populations among which gene flow has been restricted to variable extents. These populations were associated with distinct regions: the south shore of the St. Lawrence estuary (I), Chaleur Bay – Miramichi River (II), the north shore of the St. Lawrence lower estuary (III), and the north shore of the St. Lawrence middle estuary – Saguenay Fjord (IV). Mild but significant heterogeneity in the frequency distribution of genotypes was found within some of these populations, suggesting incomplete panmixia. Namely, smelt from the Matane sample, which represented the only collection site on the south shore of the lower estuary, differed significantly from the other smelt within population I, as did the Miramichi River smelt within group II. These observations may be interpreted from two perspectives. From a population genetic perspective, this suggests that even though effective migration between these sites and others from the same region has been much more important than among regions, it may have remained sufficiently small to allow significant divergence in mtDNA gene frequencies. From an ecological perspective, this suggests that short-term fluctuations in abundance at these sites could depend more on local recruitment and mortality than immigration and emigration. The Saint-Siméon sample also differed slightly within group IV. In this case, however, the distinction was most likely due to the fact that this sample was obtained from a fall commercial fishery and represented a mixture of smelt from the north- and south-shore populations of the middle estuary (Bernatchez et al. 1995).

Congruence in mtDNA and phenotypic population structure

Genetic subdivisions observed in this study reinforced earlier interpretations of smelt population structure based on preliminary genetic analyses and variation in biological characters.

For example, our data confirmed the hypothesis that smelt from the south shore of the St. Lawrence estuary comprised a population distinct from that in Chaleur Bay (Baby et al. 1991). The four populations identified by patterns of mtDNA diversity were also highly congruent with the classification of anadromous smelt into four regional groups (Chaleur Bay, south shore of St. Lawrence estuary, north shore of St. Lawrence lower estuary, and Saguenay Fjord) as proposed by Fréchet et al. (1983*a*, 1983*b*). Our study also indicated that the Saguenay Fjord group identified by these authors is not limited to the Saguenay River but extends eastward to the north shore of the St. Lawrence middle estuary. Therefore, the analysis of mtDNA variation confirmed that phenotypic variation in anadromous smelt was not solely due to the effect of variable expression of a single gene pool in the face of locally varying environmental factors but also demonstrated genetic divergence.

Smelt population structure and the member–vagrant hypothesis

Significant genetic subdivision was observed among all smelt populations, which suggested that gene flow has been insufficient to counteract their divergence through genetic drift and (or) selection. Our results, therefore, imply that smelt populations have the potential to develop local adaptations and follow distinct evolutionary trajectories. Consequently, the demonstration that the population genetic structure observed in smelt is primarily determined by the number of distinct environmental settings permitting retention of early life-history stages would provide support for the hypothesis that ecological processes inferred by the member–vagrant hypothesis (Sinclair 1988) may have evolutionary consequences for the process of population divergence. Two conditions must be met to make such an inference. First, it must be shown that the number of genetically distinct populations is more dependent on the number of suitable habitats permitting early life-history stage retention and survival than the number of spawning sites. Secondly, evidence must be provided that the extent of gene flow among populations is primarily affected by levels of dispersal of young rather than older life-history stages.

Our results provided some evidence that ecological factors affecting the distribution of young life-history stages may be important in shaping smelt genetic population structure. Thus, they clearly indicated that population richness was not primarily determined by the number of spawning sites, as illustrated by the general lack of genetic subdivision among smelt obtained from different sites within the four populations identified. There is also support for an association between genetic subdivisions and distinct retention zones for at least two of these populations. Studies of larval smelt provided sound evidence that the St. Lawrence middle estuary represents a suitable habitat within which drifting larvae from spawning tributaries located on the estuary of the south shore are retained by active vertical displacements in combination with the circulation system (Ouellet and Dodson 1985; Laprise and Dodson 1989*a*, 1989*b*). There is also evidence that Chaleur Bay constitutes a distinct oceanographic setting (Legendre and Watt 1970; El-Sabh and Ingram 1992) that could potentially retain smelt larvae associated with that system, although the larval ecology of that population has not been investigated.

Evidence that the extent of gene flow is primarily affected by levels of dispersal of young rather than older life-history stages can be inferred by comparing the species ontogenic constraints to movements within the studied area. Regardless of life-history stages, gene flow among smelt populations must primarily be governed by ecological and (or) genetic factors as there are no physical barriers (such as land or insufficient water depth) that can effectively separate them. There are no apparent physiological or environmental barriers that could limit juvenile and adult smelt dispersal among the different regions covered in this study. Thus, suitable salinity and temperature conditions for the older life-history stages of smelt can be found all over the estuary and the Gulf of St. Lawrence (Scott and Scott 1988; Nellbring 1989). Furthermore, tagging studies have shown that smelt are capable of migrating long distances in a relatively short period. For instance, Magnin and Beaulieu (1965) reported that the recovery distance for smelt tagged in the St. Lawrence estuary averaged 150 km, while several individuals were recaptured more than 300 km away. These authors also reported displacement rates up to 42 km/day, and no clear pattern in the frequency of recaptures upstream or downstream of tagging sites. This suggests that geographic distance is not an important limiting factor for smelt movements among populations. This conclusion is supported by the general lack of correlation observed between geographic and genetic distances in the present study. Altogether, these observations suggest that the dispersal capability of older smelt life-history stages is not a major constraint to gene flow among the populations surveyed.

In contrast, there are several indications that the potential for larval dispersal among populations may be highly controlled by environmental constraints. Ecological studies have shown that anadromous smelt larvae are typically associated with low salinity, being generally rare where it exceeds 20 g/kg (Able 1978; Laprise and Dodson 1989*a*; de Lafontaine 1990). This suggests that zones of high salinity may hamper survival of smelt larvae that diffuse from retention zones. Such conditions are typically found in the Laurentian channel of the lower estuary, which may limit dispersal between populations associated with both shores. Similarly, high salinity also characterizes coastal waters on the south shore of the estuary downstream of Matane (Koutitonsky and Bugden 1991), which could limit dispersal between the St. Lawrence estuary and Chaleur Bay populations.

Other oceanographic features may also restrict transport of vagrant larvae among populations. For example, the mouth of the Saguenay River (Fig. 1) is characterized by the presence of two distinct water masses, rapid depth changes, and tidally induced upwelling that creates fronts (steep gradients of physical conditions) that could prevent transport and diffusion between smelt populations of the middle and lower estuary (Ingram and El-Sabh 1990). The effect of environmental constraints on gene flow mediated by larval dispersion is also suggested by comparing levels of population differentiation in relationships with water circulation patterns. The general current pattern in the St. Lawrence lower estuary is characterized by an intense south-shore buoyant jet, and a less intense coastal current that flows seaward and parallel to the north shore. The latter current flows southward across the mouth of the estuary to join the south-shore jet and a strong coastal current, the Gaspé current, which flows along the coast of the Gaspé peninsula, towards Chaleur Bay. This circulation

pattern apparently offers little possibility for larval diffusion between populations located on the two shores of the lower estuary. In contrast, it could potentially favour larval drift along the south shore of the Gaspé peninsula down to Chaleur Bay. This may partly explain why the estimated number of female migrants per generation between the St. Lawrence south shore and Chaleur Bay populations ($N_em_f = 15$) was five times higher than that observed between the south- and north-shore populations in the lower estuary ($N_em_f = 3.3$) despite the fact that much shorter distances separate these latter two (e.g., 75 km between Matane and Baie-Comeau versus 300 km between Matane and Grande-Rivière).

Altogether, these observations strongly suggest that the number of genetically distinct populations found in anadromous smelt is more dependent on the number of suitable habitats for retention of early life-history stages than the number of spawning sites. They also suggest that gene flow may be more constrained by the probability of transport and diffusion of young life-history stages among populations than the potential for movements of older stages. Thus, ecological processes inferred by the member–vagrant hypothesis to explain the distribution of young life-history stages may be a primary factor controlling population genetic diversity in anadromous smelt. Additional factors, however, must be invoked to explain the strong genetic subdivision observed among some populations.

Historical events and population divergence

The most salient feature of this study was the demonstration of pronounced genetic differentiation between smelt samples collected on the north shore of the St. Lawrence middle estuary and the Saguenay Fjord (population IV) and all other populations. Thus, estimates of genetic subdivision involving pairwise comparisons of population IV were always more significant than for comparisons among other populations (averaged $F_{st} = 0.153$ versus 0.061), regardless of the geographic distance involved. This is best reflected by the genetic subdivision between populations from the south (I) and north (IV) shores of the middle estuary, which was approximately six times more pronounced than that between the south shore and Chaleur Bay (II) populations despite the much closer geographic proximity of the samples from the north and south shores (approximately 20 km) compared with the distance separating the middle estuary and Chaleur Bay (approximately 700 km). As reflected by estimates of the number of effective female migrants per generation, this suggests that historical and (or) contemporary gene flow has been more restricted between populations found on both shores of the middle estuary than between the south-shore and Chaleur Bay populations. This genetic structure cannot be solely explained by the effect of ecological processes inferred by the member–vagrant hypothesis. Thus, the middle estuary is generally recognized as one oceanographic setting characterized by a well-mixed zone in the upstream section and partially stratified water in the downstream part (El Sabh 1988) within which smelt larvae are retained by active and passive processes (Laprise and Dodson 1989*a*, 1989*b*). Furthermore, strong transversal currents related to the general cyclonic circulation pattern of the middle estuary (Ouellet and Trump 1979) promote larval transport across the estuary. Consequently, preliminary analyses of the spatial distribution of young life-history stages suggests important mixing of larvae and fry of both populations (Bernatchez et al. 1995, D. Pigeon, Département de biologie, Université Laval, unpublished data). In such a situation, the member–vagrant hypothesis would predict the existence of a single population in that system.

This suggests that the genetic differentiation between north- and south-shore smelt populations may primarily be due to reproductive isolation imposed by genetic rather than ecological constraints. Unlike the distinction among other populations that is primarily based on differential frequency distribution of haplotypes belonging to mtDNA phylogenetic group A, the uniqueness of population IV is due to the predominance of phylogenetic group B haplotypes. The phylogenetic basis of this differentiation is further illustrated by comparing F_{st} and Phi-CT estimates, the latter taking into account both the differences in haplotype frequency distribution and their nucleotide divergence. Thus, values obtained for both estimates were comparable in pairwise comparisons not involving population IV (mean $F_{st} = 0.080$, mean Phi-CT = 0.061), which suggests a weak phylogenetic component to their differentiation. In contrast, Phi-CT estimates were about three times higher than F_{st} values in comparisons including population IV (mean $F_{st} = 0.153$, mean Phi-CT = 0.440), which reflects a strong phylogenetic component associated with the differential distribution of mtDNA groups A and B. Therefore, the genetic differentiation of population IV from all others may more likely be explained by historical rather than contemporary factors. On the basis of the net sequence divergence estimate between mtDNA groups A and B (0.7%) and the range of mutation rates reported for the fish mitochondrial genome (Meyer 1994), it is very unlikely that they were derived from a common ancestor in postglacial times. An alternative explanation may be that mtDNA groups A and B are associated with two smelt assemblages that evolved in separate glacial refugia and subsequently recolonized the contemporary range of the species. This scenario is partly suggested by the differential pattern of distribution of the two groups in previous studies of mtDNA variation in smelt (Taylor and Bentzen 1993*a*), although a more complete phylogeographic study of the species is required to test this hypothesis. If true, this phylogenetic hypothesis implies that the St. Lawrence estuary represents a zone of secondary contact of two glacial races of smelt that have developed some biological barriers to gene flow. The possibility for the development of reproductive isolation mechanisms between intraspecific groups derived from allopatric divergence in glacial times has previously been documented for other fishes, namely the whitefish (*Coregonus* sp.) species complex (Bernatchez and Dodson 1990; Bernatchez et al. 1996).

In summary, the pattern of mtDNA diversity observed in anadromous smelt supports the hypothesis that the species' genetic population structure is primarily determined by the number of environmental settings controlling the distribution and dispersal of early life-history stages. This implies that ecological processes inferred by the member–vagrant hypothesis (Sinclair 1988) to explain geographic and phenotypic discreteness in aquatic organisms may also translate into genetic divergence, and therefore may have evolutionary consequences for the process of population divergence. Our results also indicated that biogeographic events probably have played a major role in shaping the population genetic diversity of anadromous smelt. Our study therefore illustrates the importance

of considering the interplay between the life-history characteristics of a species, its genetic diversity, and environmental constraints in a historical perspective to better understand its evolutionary ecology.

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References

Able, K.W. 1978. Ichthyoplankton of the St. Lawrence estuary: composition, distribution, and abundance. J. Fish. Res. Board Can. **35**: 1518–1531.

Avise, J.C., Helfman, G.S., Saunders, S., Stanton, N.C., and Hales, L. 1986. Mitochondrial DNA differentiation in North Atlantic eels: population genetic consequences of an unusual life history pattern. Proc. Natl. Acad. Sci. U.S.A. **83**: 4350–4354.

Baby, M.-C., Bernatchez, L., and Dodson, J.J. 1991. Genetic structure and relationships among anadromous and landlocked populations of rainbow smelt (*Osmerus mordax*, Mitchill) as revealed by mitchondrial DNA restriction analysis. J. Fish Biol. **39**(Suppl. A): 61–68.

Bentzen, P., Brown, G.C., and Leggett, W.C. 1989. Mitochondrial DNA polymorphism, population structure and life history variation in American shad (*Alosa sapidissima*). Can. J. Fish. Aquat. Sci. **46**: 1446–1454.

Bernatchez, L. 1995. Détermination du degré de différenciation génétique entre échantillons d'Éperlan arc-en-ciel (*Osmerus mordax* Mitchill) provenant de la pêcherie hivernale de Miguasha et de sites éloignés de la Baie-des-Chaleurs. Ministère de l'Environnement et de la Faune, Direction régionale Gaspésie – Iles-de-la-Madeleine, Service de l'aménagement et de l'exploitation de la faune, New-Richmond, and Institut national de la recherche scientifique (INRS-Eau).

Bernatchez, L., and Dodson, J.J. 1990. Allopatric origin of sympatric populations of lake whitefish (*Coregonus clupeaformis*) revealed by mitochondrial DNA restriction analysis. Evolution, **44**: 1263–1271.

Bernatchez, L., Savard, L., Dodson, J.J., and Pallotta, D. 1988. Mitochondrial DNA sequence heterogeneity among James–Hudson Bay anadromous coregonines. Finn. Fish. Res. **9**: 17–26.

Bernatchez, L., Martin, S., Bernier, A., Tremblay, S., Trencia, G., Verreault, G., and Vigneault, Y. 1995. Conséquences de la structure génétique de l'Éperlan arc-en-ciel (*Osmerus mordax)* pour la réhabilitation de l'espèce dans l'estuaire du Saint-Laurent. Rapport Saint-Laurent Vision 2000.

Bernatchez, L., Vuorinen, J.A., Bodaly, R.A., and Dodson, J.J. 1996. Genetic evidence for reproductive isolation and multiple origins of sympatric trophic ecotypes of whitefish (*Coregonus*). Evolution, **50**: 624–635.

Billington, N., and Hebert, P.D.N. 1991. Mitochondrial DNA diversity in fishes and its implications for introductions. Can. J. Fish. Aquat. Sci. **48**(Suppl. 1): 80–94.

Bowen, B.W., and Avise, J.C. 1990. Genetic structure of Atlantic and Gulf of Mexico populations of sea bass, menhaden, and sturgeon: influence of zoogeographic factors and life-history patterns. Mar. Biol. (Berlin), **107**: 371–381.

Carr, S.M., and Marshall, H.D. 1991. Detection of intraspecific sequence variation in the mitochondrial cytochrome *b* gene of Atlantic cod (*Gadus morhua*) by the polymerase chain reaction. Can. J. Fish. Aquat. Sci. **48**: 48–52.

Chapman, R.W., and Powers, D.A. 1984. A technique for the rapid isolation of mitochondrial DNA from fishes. Technical report. Maryland Sea Grant Program.

Chapman, R.W. 1990. Mitochondrial DNA analysis of striped bass populations in Chesapeake Bay. Copeia, **335**: 355–366.

Davidson, W.S., Birt, T.P., and Green, J.M. 1989. A review of genetic variation in Atlantic salmon, *Salmo salar* L., and its importance for stock identification, enhancement programs and aquaculture. J. Fish Biol. **34**: 547–560.

de Lafontaine, Y. 1990. Ichthyoplankton communities in the St. Lawrence Estuary: composition and dynamics. *In* Oceanography of a large-scale estuarine system. *Edited by* M.I. El-Sabh and N. Silverberg. Springer-Verlag, New York. pp. 321–343.

Dodson, J.J., Carscadden, J.E., Bernatchez, L.L., and Colombani, F. 1991. Relationship between spawning mode and phylogeographic structure in mitochondrial DNA of North Atlantic capelin (*Mallotus villosus*) populations. Mar. Ecol. Prog. Ser. **76**: 103–113.

El-Sabh, M.I. 1988. Physical oceanography of the St. Lawrence estuary. *In* Hydrodynamics of estuaries. Vol. II. Estuarine case studies. *Edited by* B. Kjerfve. CRC Press, Inc., Boca Raton, Fla. pp. 61–78.

El-Sabh, M.I., and Ingram, G.B. 1992. Large-scale hydrodynamic studies of Baie des Chaleurs in support of scallop research. *In* Proceedings of the 26th Annual Conference of the Canadian Meteorological and Oceanographical Society, Québec, Que.

Excoffier, L., Smouse, P.E., and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, **131**: 479–491.

Felsenstein, J. 1993. PHYLIP (phylogeny inference package). Version 3.5c. Department of Genetics, SK.-50, University of Washington, Seattle, Wash.

Ferguson, M. 1994. The role of molecular genetic markers in the management of cultured fishes. Rev. Fish Biol. Fish. **4**: 351–373.

Fréchet, A., Dodson, J.J., and Powles, H. 1983*a*. Use of variation in biological characters for the classification of anadromous rainbow smelt (*Osmerus mordax*) groups. Can. J. Fish. Aquat. Sci. **40**: 718–727.

Fréchet, A., Dodson, J.J., and Powles, H. 1983*b*. Les parasites de l'Éperlan d'Amérique (*Osmerus mordax*) anadrome du Québec et leur utilité comme étiquettes biologiques. Can. J. Zool. **61**: 621–626.

Iles, T.D., and Sinclair, M. 1982. Atlantic herring: stock discreteness and abundance. Science (Washington, D.C.), **215**: 627–633.

Ingram, R.G., and El-Sabh, M.I. 1990. Fronts and mesoscale features in the St. Lawrence estuary. *In* Oceanography of a large-scale estuarine system. *Edited by* M.I. El-Sabh and N. Silverberg. Springer-Verlag, New York. pp. 71–93.

Kornfield, I., and Bogdanowicz, S.M. 1987. Differentiation of mitochondrial DNA in Atlantic herring, *Clupea harengus*. Fish. Bull. **85**: 561–568.

Koutitonsky, V.G., and Bugden, G.L. 1991.The physical oceanography of the Gulf of St. Lawrence: a review with emphasis on the synoptic variability of the motion. *In* The Gulf of St. Lawrence: small ocean or big estuary? *Edited by* J.-C. Therriault. Can. Spec. Publ. Fish. Aquat. Sci. No. 113. pp. 57–90.

Laprise, R., and Dodson, J.J. 1989*a*. Ontogeny and importance of tidal vertical migrations in the retention of larval smelt *Osmerus*

mordax in a well-mixed estuary. Mar. Ecol. Prog. Ser. **55**: 101–111.

Laprise, R., and Dodson, J.J. 1989*b*. Ontogenetic changes in the longitudinal distribution of two species of larval fish in a turbid well-mixed estuary. J. Fish Biol. **35**(Suppl. A): 39–47.

Legendre, L., and Watt, D. 1970. The production of primary production relative to a cyclonic gyre in Baie des Chaleurs. Mar. Biol. (Berlin), **7**: 167–170.

Magnin, E., and Beaulieu, G. 1965. Quelques données sur la biologie de l'Éperlan *Osmerus eperlanus mordax* (Mitchill) du Saint-Laurent. Nat. Can. (Que.), **92**: 81–105.

McElroy, D., Moran, P.E., Bermingham, E., and Kornfield, I. 1992. REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. J. Hered. **83**: 157–158.

Meyer, A. 1994. Molecular phylogenetic studies of fish. *In* Genetics and evolution of aquatic organisms. *Edited by* A.R. Beaumont. Chapman & Hall, London. pp. 219–248.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, **89**: 583–590.

Nellbring, S. 1989. The ecology of smelts (genus *Osmerus*): a literature review. Nord. J. Freshwater Res. **65**: 116–145.

Ouellet, P., and Dodson, J.J. 1985. Dispersion and retention of anadromous rainbow smelt (*Osmerus mordax*) larvae in the middle estuary of the St. Lawrence River. Can. J. Fish. Aquat. Sci. **42**: 332–341.

Ouellet, Y., and Trump, C. 1979. La circulation hydrodynamique dans la zone de mélange estuarienne du Saint-Laurent. Nat. Can. (Que.), **106**: 13–26.

Ovenden, J.R. 1990. Mitochondrial DNA and marine stock assessment: a review. Aust. J. Mar. Freshwater Res. **41**: 835–853.

Roff, D.A., and Bentzen, P. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. Mol. Biol. Evol. **6**: 539–545.

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. J. Fish. Res. Board Can. Bull. No. 184.

Scott, W.B., and Scott, M.G. 1988. Atlantic fishes of Canada. Can. Bull. Fish. Aquat. Sci. No. 219.

Sinclair, M. 1988. Marine populations. An essay on population regulation and speciation. University of Washington Press, Seattle, Wash.

Sinclair, M., and Iles, T.D. 1988. Population richness of marine fish species. Aquat. Living Resour. **1**: 71–83.

Sinclair, M., and Iles, T.D. 1989. Population regulation and speciation in the oceans. J. Cons. Cons. Int. Explor. Mer, **45**: 165–175.

Sneath, P.H.A., and Sokal, R.R. 1973. Numerical taxonomy. Freeman Press, San Francisco.

Sokal, R.R., and Rohlf, F.J. 1981. Biometry. W.H. Freeman and Company, New York.

Stott, W., Ferguson, M.M., and Tallman, R.F. 1992. Genetic population structure of American plaice (*Hippoglossoides platessoides*) from the Gulf of St. Lawrence, Canada. Can. J. Fish. Aquat. Sci. **49**: 2538–2545.

Taylor, E.B., and Bentzen, P. 1993*a*. Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Osmerus*) in northeastern North America. Evolution, **47**: 813–832.

Taylor, E.B., and Bentzen, P. 1993*b*. Molecular genetic evidence for reproductive isolation between sympatric populations of smelt *Osmerus* in Lake Utopia, south-western New Brunswick, Canada. Mol. Ecol. **2**: 345–357.

Taylor, E.B., and Dodson, J.J. 1994. A molecular analysis of relationships and biogeography within a species complex of holarctic fish (genus *Osmerus*). Mol. Ecol. **3**: 235–248.

Wirgin, I., Maceda, L., Waldman, J.R., and Crittenden, R.N. 1993. Use of mitochondrial DNA polymorphisms to estimate the relative contributions of the Hudson River and Chesapeake Bay striped bass stock to the mixed Atlantic coastal fishery. Trans. Am. Fish. Soc. **122**: 669–684.

Zwanenburg, K.C.T., Bentzen, P., and Wright, J.M. 1992. Mitochondrial DNA differentiation in western North Atlantic populations of haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci. **49**: 2527–2537.

Timing of imprinting to natural and artificial odors by coho salmon (*Oncorhynchus kisutch*)

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Abstract: Juvenile salmon learn odors associated with their home stream before seaward migration and use these odor memories for homing as adults. Experimental evidence has indicated that this olfactory imprinting occurs during a sensitive period associated with surges in plasma thyroxine levels during parr–smolt transformation; however, many natural migration patterns clearly indicate that home stream learning must occur before the sensitive period at parr–smolt transformation. To examine the timing of olfactory imprinting, we exposed hatchery-reared coho salmon (*Oncorhynchus kisutch*) to either natural odors or an artificial odorant (β-phenylethyl alcohol) at specific developmental stages and tested their adult behavioral responses to these odors. Our results confirmed the importance of the parr–smolt transformation as a sensitive period for olfactory imprinting: only salmon exposed to β-phenylethyl alcohol at the smolt stage demonstrated an increased attraction to this odorant as adults. We found little evidence that salmon learned homing clues prior to the smolt stage. Results further suggested that migration, and the associated stimuli, may also be important for optimal imprinting. Therefore, the absence of presmolt learning in this study may be due, in part, to the stable environment and restrictions on migration associated with hatchery rearing.

Résumé : Avant leur migration vers la mer, les juvéniles de saumons mémorisent les odeurs associées à leur cours d'eau natal et utilisent ces souvenirs pour leur remontée à l'âge adulte. On a démontré expérimentalement que cette imprégnation olfactive survient au cours d'une période sensible associée à des hausses momentanées des teneurs en thyroxine plasmique au cours de leur transformation de tacon en smolt. Toutefois, les profils de migration de nombreuses populations de saumons sauvages indiquent clairement que la mémorisation des odeurs du cours d'eau natal doit survenir avant la période de sensibilité de la transformation de tacon en smolt. Afin d'étudier la chronologie de l'imprégnation olfactive, nous avons exposé des saumons cohos (*Oncorhynchus kisutch*) élevés en pisciculture soit à des odeurs naturelles, soit à un arôme artificiel (alcool β-phénéthylique) lors de stades spécifiques du développement, et nous avons testé les réponses comportementales de poissons adultes à ces odeurs. Nos résultats ont confirmé l'importance de la période de la transformation de tacon en smolt comme période de sensibilisation par imprégnation olfactive : seuls les saumons exposés à l'alcool β-phénéthylique au stade de smolt présentaient à l'âge adulte un attrait prononcé pour cet arôme. Très peu de résultats indiquaient que le saumon mémorisait des caractéristiques de son cours d'eau natal avant le stade de smolt. En outre, les résultats suggéraient que la migration et les stimuli associés à celle-ci pourraient être aussi importants que l'imprégnation olfactive. Par conséquent, l'absence de cas de mémorisation précédant le stade de smolt dans cette étude peut s'expliquer en partie par le milieu stable et les entraves à la migration caractérisant l'élevage en pisciculture.
[Traduit par la Rédaction]

Introduction

Salmonids are well known for their ability to return to their river of origin to spawn. Prior to their seaward migration, juvenile salmon are thought to learn site-specific odors emanating from the vegetation and soil of their natal stream and later use these odor memories for homing (Wisby and Hasler 1954; Hasler and Scholz 1983). This imprinting phenomenon was elegantly demonstrated by Hasler and his colleagues, who showed that juvenile coho salmon (*Oncorhynchus kisutch*) exposed to synthetic chemicals, such as morpholine or β-phenylethyl alcohol (PEA), could be attracted into an unfamiliar stream scented with one of these chemicals during their spawning migration 1.5 years later (Cooper et al. 1976; Scholz et al. 1976). Subsequent laboratory experiments led Hasler and Scholz (1983) to hypothesize that juvenile salmon imprint on home-stream odors during a single sensitive period of development, the parr–smolt transformation (PST). This life stage is characterized by physiological, morphological, and behavioral changes that ready salmon for life at sea. Many of these changes are associated with surges in the hormone thyroxine that occur during smolting (Dickhoff et al. 1978; Dickhoff and Sullivan 1987) and these high thyroxine levels

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are thought to be involved in olfactory learning (Hasler and Scholz 1983; Morin et al. 1989*a*, 1989*b*).

While experimental evidence with both hatchery- and laboratory-reared fish indicates that the PST is important for imprinting, the complex freshwater migration patterns of wild salmon suggest that olfactory imprinting can also occur prior to smolting. For example, sockeye salmon (*Oncorhynchus nerka*) fry typically emerge from their natal stream gravel and immediately migrate downstream to a lake where they live for 1 or 2 years before smolting and migrating to sea. During their homing migration, adults migrate past the outlet stream and lake where they smolted and return to their natal stream to spawn (e.g., Varnavskaya et al. 1994). Similarly, coho and chinook salmon (*Oncorhynchus tshawytscha*) often make extensive migrations in freshwater prior to the PST (e.g., Peterson 1982; Murray and Rosenau 1989; Scrivener et al. 1994) yet still home to their natal sites.

The natural migratory patterns of juvenile salmon in freshwater and subsequent natal stream homing led Harden Jones (1968) and Brannon (1982) to propose that juvenile salmon learn a series of olfactory way points as they migrate through freshwater to the sea and later retrace this odor sequence as adults. Such a scenario might explain the homing patterns observed in salmon released from locations other than their rearing site. Juvenile salmon transported to a different river system or long distances downstream within the same river system tend to return as adults to their release site (e.g., Donaldson and Allen 1957; Jensen and Duncan 1971; Solazzi et al. 1991; reviewed in Lister et al. 1981). On the other hand, the closer the release site is to the rearing site, the higher the percentage of salmon returning to their rearing site as adults (Lister et al. 1981; Johnson et al. 1990). This suggests that salmon may initially return to their site of release, but if they can then detect the odors of their rearing site they will migrate there. This hypothesis is supported by the finding that coho salmon smolts displaced downstream prior to release can learn the odor characteristics of both their rearing site and their downriver release site (Quinn et al. 1989).

Most studies investigating a sensitive period for homestream imprinting in salmonids have focused on the ability of juveniles to learn site-specific odors either just prior to, or during PST (reviewed in Hasler and Scholz 1983). However, juvenile salmon learn odors during earlier developmental periods. Morphological evidence suggests that the salmonid olfactory system is functional as early as hatching (Brannon 1972; Hara and Zielinski 1989). Soon after emergence, salmon are able to learn odors associated with specific habitats (e.g., lake versus river water; Brannon 1972; Bodznick 1978) and odors from other fishes (e.g., Quinn and Hara 1986; Courtenay 1989). Courtenay and Morin, cited in Courtenay (1989), demonstrated that juvenile coho salmon exposed to morpholine shortly after or even before hatching retained a memory of this odorant over a year later. However, it is not known whether odor memories established in early development are maintained until maturity and used for homing. In the present study, we examined the timing of olfactory imprinting for homing by exposing juvenile coho salmon to natural odors or to an artificial odorant, PEA, as either embryos, parr, or smolts or continuously throughout their lives. We subsequently tested whether these fish retained memories of these odors by examining the behavioral responses of mature and immature fish 17 months later.

Materials and methods

Fish and experimental approach

On November 30 and December 1, 1987, we collected and fertilized 70 000 eggs from adult coho salmon that had returned to the University of Washington hatchery (UWH) (Figs. 1A, 1B). The resulting embryos were subsequently divided into the experimental lots described below. Salmon were reared under an accelerated feeding and temperature regime that allowed them to smolt in the first spring after hatching (Brannon et al. 1982). Stages of development during the PST were determined by changes in morphology and coloration, and the ability of fish to maintain blood plasma sodium levels within 5% during a seawater challenge (Clarke and Blackburn 1977; Sullivan 1986; Dittman et al. 1994). Between May 9 and 20, salmon were dark with distinct parr marks and were unable to maintain plasma sodium levels within 5% (freshwater exposure: 155.9 ± 0.8 mequiv. Na^+/L plasma, $N = 8$; seawater exposure: 179.7 ± 2.7 mequiv. Na^+/L plasma, $N = 12$). Thus, juvenile salmon exposed to odors during this period were parr. Between June 15 and 25, 1988, salmon were elongate and silvery and were able to maintain plasma sodium levels after seawater exposure (freshwater exposure: 152.9 ± 1.2 mequiv. Na^+/L plasma, $N = 10$; seawater exposure: 159.0 ± 1.7 mequiv. Na^+/L plasma, $N = 8$). Thus, juvenile salmon exposed to odors during this period were smolts.

Three sets of experiments were conducted to test the ability of juvenile coho salmon to imprint during distinct developmental stages. First, we assessed the ability of salmon to learn the artificial odorant PEA as a homing clue by exposing fish to PEA at the alevin, parr, or smolt stages and later examining the responses of mature fish to water scented with this odorant. Second, we assessed the ability of juvenile coho salmon to learn natural site-specific odors by manipulating the rearing water that they experienced during the embryo, parr, and smolt stages prior to release and examining their subsequent homing patterns as adults. Third, we compared the learning and homing ability of salmon exposed to a single water source throughout their lives (embryo to adult stages) versus salmon exposed to this water source only through PST.

Exposure to PEA at specific life stages

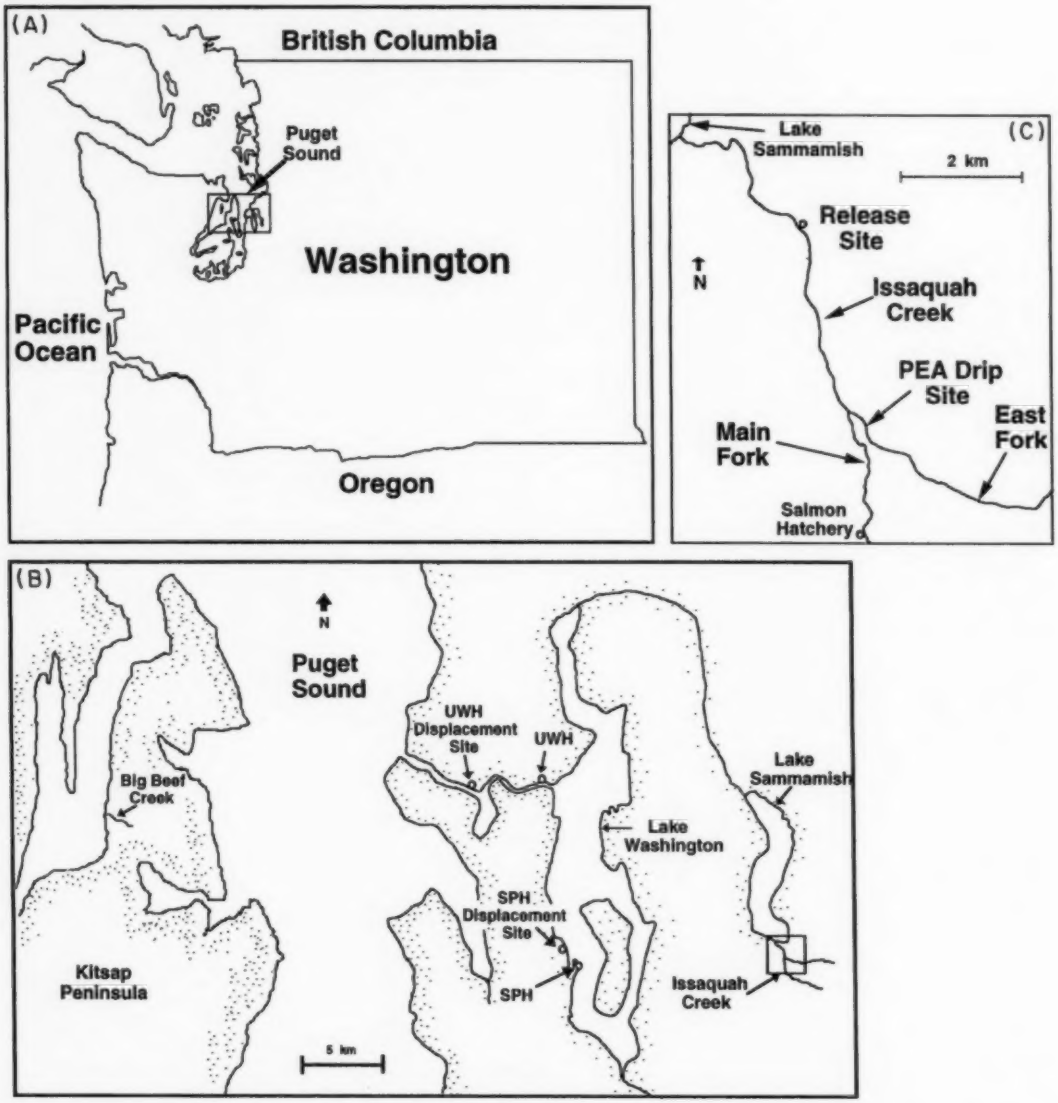
The ability of coho salmon to learn PEA as a homing clue was assessed by exposing juveniles to PEA at specific developmental stages and later examining the responses of mature fish to this odorant. Twenty thousand fertilized eggs were divided into four lots and reared separately in UWH water until hatching was completed in late January, 1988. The alevin, parr, and smolt exposure groups were continuously exposed to 10^{-7} M PEA (Sigma Chemical Co., St. Louis, Mo.) from January 29 to February 20, from May 9 to 20, and from June 15 to 24, respectively. A fourth group was maintained as a PEA-naive control. After PST, the members of each group were given a distinct fin clip, transferred to the Seward Park hatchery (SPH) (Figs. 1A, 1B), and reared communally until they matured in fall 1989. Mature fish were tested for responsiveness to PEA in two separate controlled field studies described below.

Big Beef Creek experiment

PEA recognition experiments were conducted in a two-choice maze constructed in a controlled-flow spawning channel located at the University of Washington Big Beef Creek Research Station, Kitsap Peninsula, Washington (Fig. 1). Big Beef Creek is outside the Lake Washington watershed and its water was unfamiliar to all experimental fish. The two-choice maze and experimental protocol have been described previously (Dittman 1994; Nevitt et al. 1994). Briefly, the maze was 39 m long with the extreme upstream section divided into two arms by a barrier extending downstream from the channel inlet. Traps in each arm allowed fish to move upstream into either arm but did not allow them to leave.

On November 11, 1989, 25 mature, tagged salmon from each

Fig. 1. (A) The location of the study sites in the Puget Sound region of Washington state, U.S.A. (B) An enlarged view of the Puget Sound region showing the location of study sites at Big Beef Creek, Issaquah Creek, the University of Washington hatchery (UWH), the Seward Park hatchery (SPH), and release locations for displacement experiments. (C) An enlarged view of the Issaquah Creek study site.



PEA exposure group were transported from SPH and released into the maze. Fish were allowed to move freely within the maze until they entered a trap. Each day, trapped individuals were removed and identified and their arm choice was recorded. Twice each week, new fish from each treatment were released into the maze to maintain the original density. We continued this procedure until 110 fish from each treatment had been tested. From November 11 to 14, no PEA was pumped into the maze and fish making choices during this period were designated as PEA-absent controls. Between November 15 and December 20, PEA was continuously pumped into the inlet of arm B of the maze to give a concentration of 10^{-7} M PEA. We also tested whether immature fish of the same age and PEA exposure history (i.e., cohorts that did not mature in 1989) would respond differently to PEA than mature fish. For these experiments, 10 immature salmon from each treatment were released into the maze on December 13 and monitored as described above.

The responses of the experimental groups in the presence and absence of PEA were compared using chi-square contingency analysis. We assumed that all fish were acting independently. Fish that did not enter either trap were excluded from the analysis of arm choice but were tallied for comparison between mature and immature fish.

Issaquah Creek experiment

We also measured PEA recognition in a natural stream setting, the

confluence of Issaquah Creek and its east fork in Issaquah, Washington (Fig. 1C). Issaquah Creek flows into Lake Sammamish in the Lake Washington watershed 3.4 km below the confluence of the two streams. On the main fork, the Washington State Department of Fisheries operated a weir and salmon hatchery 1.6 km upstream from the confluence with the east fork. During this study, salmon migrating up the main fork could not pass the weir and had to enter the hatchery. No traps or weirs were located on the east fork. The flow in the main fork was approximately 8–10 times greater than in the east fork, although flows fluctuated considerably during this study.

We expected that in the absence of any familiar odors (i.e., PEA), most fish either would migrate up the larger (main) fork or would not migrate upstream at all. On October 10, 1989, 1 week before releasing fish at the study site, we began pumping PEA into the east fork to establish a concentration of 10^{-6}–10^{-7} M PEA in this creek. The PEA pump was adjusted daily to maintain this concentration throughout the study. On October 17, 100 mature fish from each of the four exposure groups (PEA-naive and alevin-, parr-, and smolt-exposure) were released into Issaquah Creek at a point 1.2 km below the confluence of the two forks. Fish from each treatment group were tagged with numbered, color-coded disc tags so that treatment group and individual identity could be determined from a distance. Fish migrating up the main fork of Issaquah Creek were monitored by recovering tagged fish in the hatchery return pond and surveying the stream from the confluence to the hatchery ladder. Fish that migrated up the east fork were monitored three times per week by surveys of the stream from the confluence to a point 4.0 km upstream. Treatment group, identification number, and location were recorded for all tagged fish sighted. Both forks were monitored from October 19 until November 12. The proportion of PEA-exposed fish choosing the PEA-scented east fork versus the main fork was compared with the proportion of PEA-naive fish choosing either fork by using Fisher's exact test.

Exposure to natural odors at specific life stages

For these experiments, salmon were reared and released at the SPH, upstream from the UWH (Fig. 1B), except for brief exposures to UWH water during specific developmental stages. Upon release as smolts in 1988, these fish migrated to sea and their return patterns were examined in 1989 and 1990. Because returning adult salmon must swim within about 100 m of the UWH to reach the SPH (Fig. 1B), we hypothesized that salmon that had learned the odors of UWH during their brief exposure there would return to UWH.

Fertilized embryos for this experiment were divided into four groups of 10 000 fish and initially reared in dechlorinated Seattle city water at the University of Washington. On January 7, 1988, the parr-, smolt-, and non-UWH exposure control groups were transferred as eyed embryos to the SPH for further incubation. Between January 7 and March 13, 1988, the embryo-exposure group was reared in UWH water (supplemented with water pumped from the hatchery's adult homing pond) and then transferred to the SPH for further rearing until release as smolts. During this exposure period, fish in the embryo-exposure group experienced UWH – homing pond water for approximately 3 weeks as unhatched embryos, 3–4 weeks as alevins, and 1–2 weeks as free-swimming fry. The parr- and smolt-exposure groups were transferred from SPH and exposed to UW hatchery – homing pond water from May 9 to 20 (parr), and from June 15 to 25 (smolts), respectively. After exposure to UWH water, fish were transferred back to SPH and reared until release. The final group, non-UWH exposure control, never experienced UWH water prior to release from SPH. Table 1 summarizes the exposure protocol for the different treatment groups. Prior to release, fish that had not smolted (as determined by color and morphology) were culled. The remaining fish in each treatment group (approximately 7000 fish per group; average weight 17.53 g) were given a distinct coded wire tag and released into Lake Washington from the SPH on July 3, 1988.

Although adult University of Washington coho salmon returned primarily in 1989, the 2nd year after their release to the Lake Washington watershed, we monitored both the UWH and SPH for returning coded-wire tagged fish from 1988 through 1990. The number and location of experimental fish captured in both the commercial and sport fisheries was also estimated from the coded wire tag data base (Markey et al. 1990, 1991; Markey and Anderson 1992).

Table 1. Summary of experimental treatments of juvenile coho salmon indicating the rearing water experienced at different developmental stages.

Developmental stage	Treatment groups			
	Embryo exposure	Parr exposure	Smolt exposure	Control
Uneyed egg	CW	CW	CW	CW
Eyed egg or alevin	UWH (Jan. 7–Mar.13)	SPH	SPH	SPH
Parr	SPH	UWH (May 9–20)	SPH	SPH
Smolt	SPH	SPH	UWH (June 15–25)	SPH

Note: Salmon were exposed to University of Washington hatchery (UWH) water as embryos, parr, or smolts or were never exposed to UWH water (control). CW refers to dechlorinated city water and SPH refers to Seward Park hatchery water. The dates in 1988 when salmon experienced UWH are also shown. All fish were released from SPH on July 3, 1988.

Odor exposure duration: embryo-to-smolt versus embryo-to-adult exposure

Typically, salmon only experience the odors of their natal sites prior to or during the PST. However, we tested whether exposure to an odor source beyond the smolt stage, without any seaward migration, would influence the tendency of mature salmon to return to that odor source. To do this, the homing tendency of salmon exposed to an odor source through the PST and released to migrate to sea was compared with that of salmon exposed to an odor source during their entire lives without any seaward migration.

Embryo-to-adult exposure

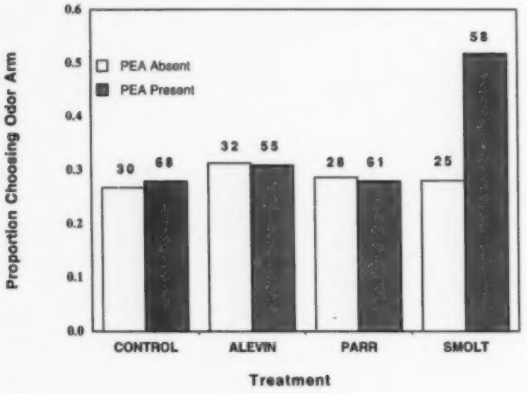
On January 7, 1988, eyed embryos were transferred from dechlorinated Seattle city water to the SPH and reared until maturity in fall 1989. Upon maturation, we tested the homing ability of these fish by displacing them a short distance downstream from SPH (Fig. 1B) and monitoring their tendency to return to the SPH. To determine whether the adult return to the hatchery was guided by olfaction rather than other sensory modalities, we made some of these fish anosmic.

On October 4, 1989, we anesthetized (MS-222; Argent) and tagged 135 mature salmon with numbered tags. Thirty-five fish were made anosmic by injecting softened wax into both nares. The wax rapidly solidified and blocked water access to the olfactory epithelium. To control for handling stress, the remaining 100 fish were treated similarly by injecting warm water into the nares (sham-operated fish). After a 48-h recovery period, the 100 sham-operated fish were transported and released from shore approximately 1.0 km north of the SPH. To ensure that anosmic fish did not simply follow sham-operated fish back to the hatchery, the anosmic fish were held for an additional 24 h before transport and release. The trap at the SPH was monitored daily for returning fish. To determine whether the anosmia operation affected survival, four anosmic and four sham-operated fish were retained at the hatchery for observation.

Embryo-to smolt exposure

For comparison with the salmon reared exclusively in the hatchery,

Fig. 2. Behavioral responses of mature coho salmon to β-phenylethyl alcohol (PEA) in the Big Beef Creek two-choice arena. Salmon were exposed to PEA at the developmental stage indicated or never experienced PEA (control). Open bars show the proportion of salmon choosing arm B in the absence of added PEA. Shaded bars show the proportion of salmon choosing arm B in the presence of PEA metered into arm B. Numbers above bars indicate the total number of fish choosing either arm A or B.



this experiment was also conducted using anosmic and sham-operated salmon displaced downstream after completing their natural homing migration. Because the SPH does not maintain a coho salmon run, these experiments were conducted at the UWH with salmon that were released as smolts and had returned to the hatchery after 1.5 years at sea. On October 26, 1988, 20 anosmic and 20 sham-operated tagged salmon were transported approximately 5 km downstream and released (Fig. 1B). The UWH homing pond was surveyed each day thereafter for tagged fish. As above, four anosmic and four sham-operated fish were maintained in the hatchery to assess the impact of the anosmia operation on health and survival.

Results

Exposure to PEA at specific life stages

In the absence of PEA, mature salmon tested in the experimental channel at Big Beef Creek entered arm A of the arena in greater numbers than arm B ($p < 0.05$ for all groups) and the proportion of fish entering arm A was the same for all PEA-exposure groups ($\chi^2 = 0.17$; $p < 0.99$; Fig. 2). When we subsequently metered 10^{-7} M PEA into the less preferred arm (arm B), the choices of salmon in the PEA-naive ($p < 0.90$), alevin-exposure ($p < 0.99$), and parr-exposure ($p < 0.95$) groups were indistinguishable from their responses in the absence of PEA (Fig. 2). In contrast, mature salmon that had been exposed to PEA as smolts showed a greater tendency to enter arm B when PEA was present than when it was absent ($\chi^2 = 3.97$; $p < 0.05$; Fig. 2). Of the 440 mature fish tested, 357 (81.1%) entered one arm or the other during the experiment. None (0.0%) of the 40 immature salmon from the four PEA-exposure groups entered either arm of the arena over the 7 days of testing. They remained primarily in the downstream section of the arena but appeared to be healthy and active.

The responses of mature PEA-exposed fish to PEA in Issaquah Creek were similar to the responses of their cohorts tested at Big Beef Creek. For all treatment groups, the majority of fish that migrated upstream were recovered in the Issaquah Creek hatchery (Table 2). However, the proportion of fish choosing the PEA-scented east fork varied according to their PEA exposure history. The proportion of mature salmon choosing the PEA-scented east fork was the same (1/26; 0.04%) for PEA-naive fish and fish exposed to PEA as alevins. On the other hand, a larger proportion of the salmon exposed to PEA as smolts migrated up the PEA-scented fork (9/33; 27.3%) compared with controls ($p = 0.018$). The parr-exposure group showed a slightly greater tendency to ascend the PEA fork (5/29; 20.8%) than controls ($p = 0.12$). The majority of fish released below the stream confluence were not recovered. Some of these fish probably moved downstream and into Lake Sammamish, where several tagged fish were recovered. All but one fish observed in the east fork was sighted on more than one occasion. Fish originally found in the east fork were never subsequently captured at the hatchery located on the main fork.

Table 2. Numbers of adult coho salmon recovered in either the β-phenylethyl alcohol (PEA)-scented east fork or the main fork (control) of Issaquah Creek after release 1.2 km below the confluence of the two streams.

	Treatment groups			
Recovery site	Control	Alevin	Parr	Smolt
Main fork (control)	25	25	24	24
East fork (PEA-scented)	1	1	5	9

Note: Salmon were exposed to 10^{-7} M PEA at three different developmental stages: alevin, parr, and smolts. Control fish never experienced PEA.

Table 3. Patterns of homing displayed by adult coho salmon reared and released at the Seward Park hatchery (SPH) except for brief exposures to University of Washington hatchery (UWH) water at one of three different developmental stages: embryo, parr, and smolt.

	Treatment			
Recovery site	Control	Embryo	Parr	Smolt
SPH	14	9	6	3
UWH	0	0	1	2
Commercial and sport fisheries	13	13	7	0

Note: Control fish never experienced UWH water.

Exposure to natural odors at specific life stages

Only salmon that experienced the UWH water as parr and smolts returned to the UWH (Table 3). Fish exposed to UWH water as embryos and control fish that never experienced UWH water returned exclusively to the SPH. Poor return rates, however, precluded statistical analysis. Of the 28 000 fish released as smolts from the SPH, only 35 mature fish returned to the SPH and UWH. The low return rates to the hatcheries were paralleled by poor returns from fishery samples

(Table 3), suggesting low survival rather than impairment of homing.

Odor exposure duration: embryo-to-smolt versus embryo-to-adult exposure

The percentage of mature salmon returning to their rearing site after displacement was lower for fish that had lived there throughout their lives than for fish released as smolts and allowed to migrate naturally. Only 18 (18.0%) of the 100 sham-operated salmon that had spent their entire lives at the SPH returned to that facility after being displaced only 1.0 km away (Fig. 3). The time to return varied between 1 and 60 days. Eighteen days after release, two of these fish were recovered 18 km away in the UWH homing pond. None of the 35 anosmic fish displaced from Seward Park returned to the SPH. No difference in survival was detected between anosmic (21.2 ± 1.5 days, mean longevity ± standard deviation (SD)) and sham-operated (20.0 ± 0.9 days) fish maintained at the hatchery during the course of this study.

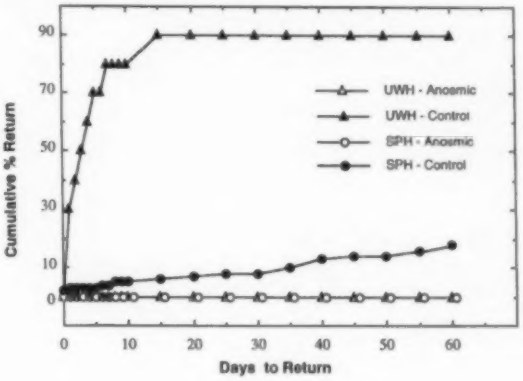
In the parallel study of salmon exposed to UWH water as juveniles only, 14 (70%) of the 20 sham-operated salmon released downstream from the UWH returned to that hatchery within 5 days and 18 salmon (90%) returned within 15 days (Fig. 3). The two salmon (10%) that did not return were captured in the commercial gill-net fishery in Lake Union. In contrast, none of the anosmic salmon returned to the UWH pond up to 60 days after release and none were reported captured in the fishery (Fig. 3). One anosmic fish was observed in the outflow of the UWH but did not enter the facility. There was no difference in survival between treatment groups for fish maintained in hatchery raceways (longevities were 14.7 ± 0.9 days for anosmic and 15.7 ± 1.0 days for sham-operated fish). Experimental manipulations were not the cause of mortality in either the lifelong or juvenile exposure experiments. The timing of death in both experiments was consistent with the natural mortality occurring after maturation and spawning.

Discussion

Timing of imprinting

Our PEA and water source exposure experiments indicated that the end of the PST (smolt stage) is a particularly important period for olfactory learning in coho salmon reared in a hatchery. In two separate experiments, mature salmon that were exposed to 10^{-7} M PEA for only 10 days as smolts displayed an increased attraction to PEA-scented water when tested 17 months later. Similarly, the return patterns of salmon exposed to UWH water as smolts, but released from the SPH, suggested that some of these fish had learned and were attracted to the UWH water. Two of the five returning fish that were exposed to UWH water as smolts were recovered at the UWH. In contrast, 100% of the 14 fish in this experiment and all 82 fish in a previous study (Brannon and Quinn 1990) that were reared and released at SPH without any UWH water exposure bypassed the UWH and returned to the SPH. Long-term olfactory learning during the smolt stage was also indicated by biochemical and electrophysiological measurements of increased peripheral olfactory sensitivity to PEA in fish from the group exposed to PEA as smolts (Dittman 1994; Nevitt et al. 1994).

Fig. 3. Cumulative frequency plot of the number of days for anosmic and control (sham operated) salmon to return to their home hatchery after release downstream from the Seward Park hatchery (SPH) and the University of Washington hatchery (UWH). Prior to displacement, SPH fish were exposed to SPH water throughout their lives while UWH fish were released from their hatchery as smolts.



While coho smolts clearly learned olfactory homing clues, there was no evidence for such learning by embryos in these experiments, even though embryos were exposed to both natural water and PEA for 2–8 weeks longer than the smolt-exposure group. Similarly, the evidence for imprinting by parr was weak. One of the seven returning fish that were exposed to UWH water as parr did return to the UWH as an adult. There was also a slightly increased tendency for salmon exposed to PEA as parr to migrate up the PEA-scented fork of Issaquah Creek. On the other hand, there was no indication of parr learning in the Big Beef Creek experiment and in all cases the homing responses of fish exposed to odors as parr were weaker than those of smolt-exposure fish and did not differ significantly from those of controls. These findings agree with previous studies implicating smolting as a critical period for olfactory imprinting (Hasler and Scholz 1983). Scholz (1980) found that coho salmon did not imprint on artificial odorants prior to PST and he proposed that olfactory imprinting in coho salmon is associated with elevated thyroid hormone levels that occur during PST. Similarly, Morin et al. (1989*a*) found that Atlantic salmon, *Salmo salar*, had an optimal period for long-term olfactory learning 21–28 days after the onset of PST, coinciding with peak levels of thyroid activity (Morin et al. 1989*b*).

Our results also suggest that zero-age coho smolts (i.e., 6 months old) provide an appropriate time-saving model system for studying the timing of imprinting. Coho salmon normally experience the PST as 18-month-old fish but under appropriate rearing conditions this process can be accelerated by 12 months (Brannon et al. 1982). The embryonic and parr stages were not affected by the accelerated rearing regime. Changes in morphology, behavior, and seawater adaptability all indicated that zero-age fish successfully smolted and were able to imprint and home accurately (Quinn et al. 1989; Brannon and Quinn 1990; the present study). There are physiological differences between zero-age and yearling smolts (e.g., reduced thyroxine surges in zero-age smolts) but these differences have no

obvious effects on imprinting and homing ability (Dittman et al. 1994).

Influence of sexual maturity

The absence of any evidence of presmolt imprinting is interesting because Courtenay (1989) found that coho salmon exposed to the artificial odorant morpholine as prehatch embryos, alevins, or fry responded to this odorant in behavioral tests 2–4 months later. Furthermore, Courtenay's fish maintained an olfactory memory for morpholine more than 1 year after exposure, as indicated by heart rate reductions in the presence of this odorant (Courtenay and Morin; cited in Courtenay 1989). The differences between our results may reflect the different developmental status of salmon tested for odor recognition (i.e., mature versus immature) because the long-term odor memory demonstrated by Courtenay (1989) may not have been associated with reproductive homing. Presmolts learn odors associated with specific habitats (e.g., lake versus river water) (Brannon 1972; Bodznick 1978) and odors from other fishes (e.g., Quinn and Hara 1986; Courtenay 1989), but it is not known whether such odor memories are important for homing.

In our Big Beef Creek experiments, immature salmon did not respond to PEA-scented water regardless of their PEA exposure history. Whether this reflects an inability to recognize PEA or a lack of motivation to respond to PEA is not clear but it does indicate that mature and immature salmon respond differently to odors learned as juveniles. Consistent with our results, Hasler and Scholz (1983) reported that only mature coho salmon responded behaviorally to imprinted odorants. Electrophysiology and heart rate conditioning experiments also indicated that olfactory sensitivity to imprinted odorants was greatest during maturation (Cooper and Hasler 1973; Hasler and Scholz 1983). It is important to note, however, that homing and maturation are not inextricably linked. Many populations of salmon migrate back to their river of origin months before maturing (e.g., spring chinook salmon and summer steelhead, *Oncorhynchus mykiss*). In these races, fish may home hundreds of kilometres upriver to the general vicinity of their natal stream, then hold in the main-stem river for several months before resuming their homing migration to their spawning site (e.g., Berman and Quinn 1991).

Presmolt imprinting

Sequential imprinting

The apparent lack of presmolt imprinting in these experiments may be due, in part, to the methods we and others have used to assess odor recognition and attraction. As part of the sequential imprinting hypothesis, Harden Jones (1968) proposed that during their homing migration, salmon retrace their seaward migration by first seeking the last odor they experienced before going to sea, then the next-to-last odor, and so on, in sequence until they arrive back at the natal site. If this is correct, the salmon in our study may have imprinted prior to smolting but did not respond to these odors because they did not first experience the odors they learned as smolts. This explanation is consistent with our PEA experiments and data from displacement experiments on the Columbia River in which salmon that had been released upriver from their rearing site as smolts migrated past the rearing site as adults and subsequently returned to the release site (Slatick et al. 1988; Pascual et al. 1995). In one Columbia River experiment, however, juvenile coho salmon released upstream of their hatchery returned as adults to their rearing hatchery, not their release site (Johnson et al. 1990). In our study, 3 of the 35 adults returning to Lake Washington after release from SPH as juveniles were recovered at the UWH but it is uncertain whether these fish entered the UWH when they first encountered UWH water, or, rather, first returned to the vicinity of the SPH and then began searching for UWH water.

Influences of thyroid hormones

Another factor that may have contributed to the lack of any observed presmolt learning is the different rearing environments experienced by hatchery-reared and wild fish and the resulting differences in thyroid hormone production. Although a direct link between elevated plasma thyroxine levels and imprinting has been questioned (Dittman et al. 1994), thyroid hormone signalling has been repeatedly implicated in olfactory imprinting (e.g., Hasler and Scholz 1983; Morin et al. 1989*a*, 1989*b*). Scholz (1980) demonstrated that presmolt salmon were unable to learn artificial odorants unless they were injected with thyroid stimulating hormone to raise thyroid hormone levels. Similarly, Morin et al. (1989*a*) found that Atlantic salmon had an optimal period for long-term olfactory learning 21–28 days after the onset of the PST, coinciding with peak levels of thyroid activity (Morin et al. 1989*b*).

Nishioka et al. (1985) hypothesized that the stable rearing conditions in most hatcheries (e.g., constant temperature, flow rate, water source) may provide insufficient environmental stimuli for optimal thyroid development. Under such conditions, hatchery fish may experience only an endogenously controlled smolting-related increase in thyroxine levels. In contrast, wild salmon experience various environmental changes that may elicit multiple thyroxine surges throughout the period of freshwater rearing (Nishioka et al. 1985). Exposure to novel water, changing lunar cycles, increased water velocity, and temperature fluctuations have all been linked to increased thyroid activity (Grau et al. 1981; Dickhoff et al. 1982; Iwamoto 1982; Youngson and Simpson 1984; Nishioka et al. 1985). Therefore, if thyroid hormones are involved in imprinting, the different thyroid hormone profiles of hatchery and wild fish might explain the apparent inconsistencies between experimental evidence obtained with hatchery-reared fish indicating that PST is a critical period for imprinting and studies with wild fish that indicate that salmon must also learn at other times.

Role of migration in imprinting

While our results indicated that the end of the PST (smolt stage) is a sensitive period for olfactory imprinting, the poor homing performance of salmon exposed throughout their lives to SPH water suggests that exposure to a water source during PST may not, in itself, be sufficient for imprinting. Only 18 of the 100 salmon displaced just 1.0 km downstream were recovered at the SPH, 2 fish were recovered at the UWH (which they had never experienced), and the time to return to the SPH was as high as 60 days. In contrast, 90% of the salmon that were released as smolts, and experienced both seaward and homing migrations, returned to their home hatchery within 15 days after displacement. As expected, none of the anosmic fish

returned to their home hatchery, confirming that the olfactory sense is required for salmon homing (Wisby and Hasler 1954). The differences in the return rates of salmon displaced from the SPH and UWH were not likely due to any inherent problems with the SPH as a return site. For example, all of the returning coho salmon reared and released from the SPH as smolts returned to that hatchery at maturity (Brannon and Quinn 1990; the present study).

The most likely factor influencing the return patterns was the different migratory experience of each group as juveniles. We hypothesize that there is a sensitive window (i.e., PST) during which salmon must migrate for optimal imprinting to occur. Thus, salmon held for their entire lives at the SPH and not allowed to migrate during the PST exhibited impaired homing compared with fish released from the UWH as smolts and allowed to migrate naturally. Salmon released from hatcheries as smolts show a greater tendency for homing than those released after the smolt stage (chinook salmon: Unwin and Quinn 1993; Pascual et al. 1995; Atlantic salmon: Hansen and Jonsson 1991). Furthermore, displacement experiments in which smolts are trucked downstream for release have indicated that while some fish return to their natal site, the majority return as adults to the area where they began their downstream migration (Slatick et al. 1988; Quinn et al. 1989).

How might migration be involved in imprinting? During migrations, swimming rates often increase and salmon move into new environments of differing water chemistry, temperatures, and flow rates. All these factors can stimulate thyroxine production (Dickhoff et al. 1982; Nishioka et al. 1985) and migrating salmon may therefore experience elevated thyroxine levels that may facilitate olfactory imprinting (see above). This may also explain why wild salmon apparently learn odors as fry (perhaps when they migrate from their incubation sites) but hatchery-reared salmon show no evidence of such learning.

Concluding remarks

In summary, our results confirm the importance of the parr–smolt transformation as a sensitive period for olfactory imprinting but suggest that exposure to a water source alone is not sufficient for optimal imprinting. There was little evidence for presmolt learning of homing clues by hatchery-reared fish. Because salmon in the wild often learn home site odors prior to smolting, the lack of any observed early learning in our study may be due to the uniformity of hatchery rearing conditions and lack of migration. Finally, our results suggest that while migration may not be absolutely required for olfactory imprinting, the combination of stimuli associated with migration and physiological changes involved in smolting may be important for optimal imprinting and homing.

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References

Berman, C.H., and Quinn, T.P. 1991. Behavioural thermoregulation and homing by spring chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), in the Yakima River. J. Fish Biol. **39**: 301–312.

Bodznick, D. 1978. Water source preferences and lakeward migration of sockeye salmon fry (*Oncorhynchus nerka*). J. Comp. Physiol. **127**: 139–146.

Brannon, E.L. 1972. Mechanisms controlling migration of sockeye salmon fry. Int. Pac. Salmon Fish. Comm. Bull. No. 21.

Brannon, E.L. 1982. Orientation mechanisms of homing salmonids. *In* Salmon and trout migratory behavior symposium. *Edited by* E.L. Brannon and E.O. Salo. School of Fisheries, University of Washington, Seattle, Wash. pp. 219–227.

Brannon, E.L., and Quinn, T.P. 1990. A field test of the pheromone hypothesis for homing by Pacific salmon. J. Chem. Ecol. **16**: 603–609.

Brannon, E.L., Feldmann, C., and Donaldson, L.R. 1982. University of Washington zero-age coho salmon smolt production. Aquaculture, **28**: 195–200.

Clarke, W.C., and Blackburn, J. 1977. A seawater challenge test to measure smolting in juvenile salmon. Tech. Rep. Fish. Mar. Serv. Can. No. 705.

Cooper, J.C., and Hasler, A.D. 1973. An electrophysiological approach to salmon homing. Fish. Res. Board. Can. Tech. Rep. No. 415.

Cooper, J.C., Scholz, A.T., Horrall, R.M., Hasler, A.D., and Madison, D.M. 1976. Experimental confirmation of the olfactory hypothesis with artificially imprinted homing coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board. Can. **33**: 703–710.

Courtenay, S.C. 1989. Learning and memory of chemosensory stimuli by underyearling coho salmon, *Oncorhynchus kisutch* Walbaum. Ph.D. thesis, University of British Columbia, Vancouver, B.C.

Dickhoff, W.W., and Sullivan, C.V. 1987. Involvement of the thyroid gland in smoltification, with special reference to metabolic and developmental processes. Am. Fish. Soc. Symp. **1**: 197–210.

Dickhoff, W.W., Folmar, L.C., and Gorbman, A. 1978. Changes in plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. **36**: 229–232.

Dickhoff, W.W., Darling, D.S., and Gorbman, A. 1982. Thyroid function during smoltification of salmonid fish. Gunma Symp. Endocrinol. **19**: 45–61.

Dittman, A.H. 1994. Behavioral and biochemical mechanisms of olfactory imprinting and homing by coho salmon. Ph.D. thesis, University of Washington, Seattle, Wash.

Dittman, A.H., Quinn, T.P., Dickhoff, W.W., and Larsen, D.A. 1994. Interactions between novel water, thyroxine and olfactory imprinting in underyearling coho salmon (*Oncorhynchus kisutch* Walbaum). Aquacult. Fish. Manage. **25**(Suppl. 2): 157–169.

Donaldson, L.R., and Allen, G.H. 1957. Return of silver salmon, *Oncorhynchus kisutch* (Walbaum) to point of release. Trans. Am. Fish. Soc. **87**: 13–22.

Grau, E.G., Dickhoff, W.W., Nishioka, R.S., Bern, H.A., and Folmar, L.C. 1981. Lunar phasing of the thyroxine surge preparatory to seawater migration of salmonid fish. Science (Washington, D.C.), **211**: 607–609.

Hansen, L.P., and Jonsson, B. 1991. The effect of timing of Atlantic salmon smolt and postsmolt release on the distribution of adult return. Aquaculture, **98**: 61–67.

Hara, T.J., and Zielinski, B. 1989. Structural and functional development of the olfactory organ in teleosts. Trans. Am. Fish. Soc. **118**: 183–194.

Harden Jones, F.R. 1968. Fish migration. Arnold, London.

Hasler, A.D., and Scholz, A.T. 1983. Olfactory imprinting and homing in salmon. Springer–Verlag, Berlin.

Iwamoto, R.N. 1982. Strain-photoperiod-temperature interactions in coho salmon: freshwater growth, smoltification and seawater adaptation. Ph.D. thesis, University of Washington, Seattle, Wash.

Jensen, A.L., and Duncan, R.N. 1971. Homing of transplanted coho salmon. Prog. Fish-Cult. **33**: 216–218.

Johnson, S.L., Solazzi, M.F., and Nickelson, T.E. 1990. Effects on survival and homing of trucking hatchery yearling coho salmon to release sites. N. Am. J. Fish. Manage. **10**: 427–433.

Lister, D.B., Hickey, D.G., and Wallace, I. 1981. Review of the effects of enhancement strategies on the homing, straying and survival of Pacific salmonids. Report prepared for the Canadian Department of Fisheries and Oceans, Vancouver, B.C.

Markey, S.L., and Anderson, L.M. 1992. 1990 results from micro-tagged salmon experimental groups. Progress Rep. No. 301. State of Washington, Department of Fisheries, Olympia, Wash.

Markey, S.L., McGowan, J.E., and Crumley, P. 1990. 1988 results from micro-tagged salmon experimental groups. Progress Rep. No. 282. State of Washington, Department of Fisheries, Olympia, Wash.

Markey, S.L., Mcgowan, J.E., and Crumley, P. 1991. 1989 results from micro-tagged salmon experimental groups. Progress Rep. No. 294. State of Washington, Department of Fisheries, Olympia, Wash.

Morin, P.-P., Dodson, J.J., and Doré, F.Y. 1989*a*. Cardiac responses to a natural odorant as evidence of a sensitive period for olfactory imprinting in young Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. **46**: 122–130.

Morin, P.-P., Dodson, J.J., and Doré, F.Y. 1989*b*. Thyroid activity concomitant with olfactory learning and heart rate changes in Atlantic salmon, *Salmo salar*, during smoltification. Can. J. Fish. Aquat. Sci. **46**: 131–136.

Murray, C.P., and Rosenau, M.L. 1989. Rearing of juvenile chinook salmon (*Oncorhynchus tshawytscha*) in non-natal tributaries of the lower Fraser River, British Columbia. Trans. Am. Fish. Soc. **118**: 284–289.

Nevitt, G.A., Dittman, A.H., Quinn, T.P., and Moody, W.J. 1994. Evidence for a peripheral olfactory memory in imprinted salmon. Proc. Natl. Acad. Sci. U.S.A. **91**: 4288–4292.

Nishioka, R.S., Young, G., Bern, H.A., Jochimsen, W., and Hiser, C. 1985. Attempts to intensify the thyroxin surge in coho and king salmon by chemical stimulation. Aquaculture, **45**: 215–225.

Pascual, M.A., Quinn, T.P., and Fuss, H. 1995. Factors affecting the homing of Columbia River hatchery-produced fall chinook salmon. Trans. Am. Fish. Soc. **124**: 308–320.

Peterson, N.P. 1982. Immigration of juvenile coho salmon (*Oncorhynchus kisutch*) into riverine ponds. Can. J. Fish. Aquat. Sci. **39**: 1308–1310.

Quinn, T.P., and Hara, T.J. 1986. Sibling recognition and olfactory sensitivity in juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Zool. **64**: 921–925.

Quinn, T.P., Brannon, E.L., and Dittman, A.H. 1989. Spatial aspects of imprinting and homing in coho salmon (*Oncorhynchus kisutch*). Fish. Bull. **87**: 769–74.

Scholz, A.T. 1980. Hormonal regulation of smolt transformation and olfactory imprinting in coho salmon. Ph.D. thesis, University of Wisconsin, Madison, Wis.

Scholz, A.T., Horrall, R.M, Cooper, J.C., and Hasler, A.D. 1976. Imprinting to chemical cues: the basis for home stream selection in salmon. Science (Washington, D.C.), **192**: 1247–1249.

Scrivener, J.C., Brown, T.G., and Andersen, B.C. 1994. Juvenile chinook salmon (*Oncorhynchus tshawytscha*) utilization of Hawks Creek, a small and nonnatal tributary of the upper Fraser River. Can. J. Fish. Aquat. Sci. **51**: 1139–1146.

Slatick, E., Gilbreath, L.G., Harmon, J.R., Bjornn, T.C., Ringe, R.R., Walch, K.A., Novotny, A.J., and Zaugg, W.S. 1988. Imprinting hatchery reared salmon and steelhead trout for homing, 1978–1983. Project No. 78-1. Report prepared for Bonneville Power Administration, Portland, Oreg.

Solazzi, M.F., Nickelson, T.E., and Johnson, S.L. 1991. Survival, contribution, and return of hatchery coho salmon (*Oncorhynchus kisutch*) released into freshwater, estuarine, and marine environments. Can. J. Fish. Aquat. Sci. **48**: 248–253.

Sullivan, C.V. 1986. Effects of the thyroid hormone triiodothyronine and the anti-thyroid drug propylthiouracil on thyroid system function, smoltification, and marine survival of coho salmon (*Oncorhynchus kisutch*). Ph.D. thesis, University of Washington, Seattle, Wash.

Unwin, M.J., and Quinn, T.P. 1993. Homing and straying patterns of chinook salmon (*Oncorhynchus tshawytscha*) from a New Zealand hatchery: spatial distribution of strays and effects of release date. Can. J. Fish. Aquat. Sci. **50**: 1168–1175.

Varnavskaya, N.V., Wood, C.C., Everett, R.J., Wilmot, R.L., Varnavsky, V.S., Midanaya, V.V., and Quinn, T.P. 1994. Genetic differentiation of subpopulations of sockeye salmon (*Oncorhynchus nerka*) within large lakes of Alaska, British Columbia and Kamchatka. Can. J. Fish. Aquat. Sci. **51**(Suppl. 1): 147–157.

Wisby, W.J., and Hasler, A.D. 1954. Effect of olfactory occlusion on migrating silver salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. **11**: 472–478.

Youngson, A.F., and Simpson, T.H. 1984. Changes in serum thyroxine levels during smolting in captive and wild Atlantic salmon, *Salmo salar* L. J. Fish Biol. **24**: 29–39.

Factors affecting the occurrence and structure of fish assemblages in isolated wetlands of the upper coastal plain, U.S.A.

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Abstract: To test a model of fish assemblage structure in isolated wetlands of the Atlantic Coastal Plain, we related the presence or absence of fishes in individual wetlands and variation in assemblage structure to environmental conditions within wetlands and to location in relation to permanent aquatic habitats. Fishes were limited to wetlands that dried infrequently, were relatively close to intermittent aquatic habitats, and had little elevation difference between the wetland and its nearest permanent aquatic habitat. Comparison of variation in assemblage structure among wetlands to null hypotheses of randomness suggested that biological interactions played a minor role in structuring these assemblages. We found no correlation between assemblage structure and wetland environment. Variation in assemblage structure was correlated with the geographic position of wetlands. Wetlands located in the upper portions of drainage basins were dominated by *Lepomis marginatus* and *Erimyzon sucetta* while wetlands located in downstream portions of drainage basins were dominated by *Acantharchus pomotis* and *Gambusia holbrooki*. Our results are consistent with a model of fish assemblage structure in which wetland drying frequency and connectivity determine the presence or absence of fishes, and differences in colonizing rates and the relative abundance of species in source pools influence variation among wetlands with fishes.

Résumé : Afin de tester un modèle de la structure des assemblages de poissons dans des terres humides isolées de la plaine côtière de l'Atlantique, nous avons établi un rapport entre, d'une part, la présence ou l'absence de poissons dans des terres humides particulières et la variation de la structure des assemblages et, d'autre part, les conditions du milieu dans ces terres humides et leur emplacement par rapport aux habitats aquatiques permanents. On ne trouvait des poissons que dans les terres humides peu sujettes à l'assèchement, relativement proches d'habitats aquatiques intermittents et ne présentant qu'une faible différence de niveau par rapport à l'habitat aquatique permanent le plus près. Une comparaison de la variation dans la structure des assemblages observée dans diverses terres humides à celle prévue par des hypothèses à effets aléatoires nuls suggérait que les interactions biologiques jouaient un rôle mineur dans la structuration de ces assemblages. On n'a observé aucune corrélation entre la structure des assemblages et le milieu des terres humides. On a corrélé la variation de la structure des assemblages à la position géographique des terres humides. Les terres humides situées dans les portions supérieures des bassins hydrographiques étaient dominées par *Lepomis marginatus* et *Erimyzon sucetta* alors que celles situées dans les portions inférieures étaient dominées par *Acantharchus pomotis* et *Gambusia holbrooki*. Nos résultats sont cohérents avec un modèle de structure des assemblages de poissons dans lequel la fréquence d'assèchement des terres humides et leur liaison avec d'autres habitats aquatiques déterminent la présence ou l'absence des poissons; de plus, les différences dans les taux de colonisation et l'abondance relative des espèces dans les habitats fournisseurs influencent les variations entre les terres humides abritant des poissons.
[Traduit par la Rédaction]

Introduction

Isolated depression wetlands are common features of the Atlantic Coastal Plain that have received little attention from ichthyologists. Some investigators have noted the occurrence of fishes in isolated wetlands (Bennett and McFarlane 1983; Schalles et al. 1989) and several authors have suggested that less than 10% of Carolina bays (a type of isolated wetland) of the Atlantic Coastal Plain have fish populations (Sharitz and Gibbons 1982; Richardson and Gibbons 1993). However, to our knowledge there has been no systematic survey of fishes in Carolina bays or isolated wetlands of the Atlantic Coastal Plain.

Under normal surface water conditions isolated depression wetlands have no surface inlet or outlet of a permanent nature. Because they are internally drained, these wetlands periodically dry. During periods of high water they may overflow into adjacent drainages, establishing temporary aquatic connections between the two systems. On the basis of geology and geomorphology or vegetation some isolated wetlands are classified as Carolina bays (Prouty 1952), shrub bogs, or pocosins (Sharitz and Gibbons 1982). Although these classifications overlap and include many isolated wetlands, they also include

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less isolated wetlands. For example, Lake Waccamaw, a large Carolina bay in North Carolina, receives continual inputs from, and continually discharges to, the Waccamaw River. For clarity we use the term isolated wetlands (or wetlands) to refer to depression wetlands that under average surface water levels are not connected to other aquatic habitats by surface waters.

In this paper we report on a survey of fishes in 63 isolated wetlands located on the Upper Coastal Plain of South Carolina: our goals were to determine the extent of fish distribution in them, and to determine the relative influence of individual wetland environments and landscape position in determining patterns of fish distribution among them. Specifically, we tested a general hierarchical model of fish assemblage structure in these habitat types. We hypothesized that fish would be limited to those wetlands that dried semiannually and were close enough to more permanent aquatic habitats to occasionally receive colonists from those habitats. We tested the null hypothesis that differences in assemblage structure between wetlands result from a random colonization process, and that assemblage structure could thus be explained by a random model. Mechanisms leading to alternative hypotheses of structured assemblages include variation in environments between wetlands, biological interactions (e.g., competition or predation), and nonrandom colonization processes (e.g., differences in the structure of the assemblage that colonists are drawn from). We define structure as variation in assemblage composition between wetlands beyond that expected by chance.

Methods

Study site

We sampled isolated wetlands on the U.S. Department of Energy's Savannah River Site (SRS), a 780-km^2 area adjacent to the Savannah River on the Upper Coastal Plain of South Carolina (Fig. 1). Information on isolated wetlands (primarily Carolina bays) in this area has been summarized by Sharitz and Gibbons (1982), Schalles et al. (1989), and Richardson and Gibbons (1993). Although many of the isolated wetlands on the SRS were disturbed by agricultural practices in the past, they have been protected since 1951. The southern portion of the SRS, along the Savannah River, lies in the Upper Coastal Plain physiographic region, and the northern portion of the SRS lies in the Sandhills physiographic region. Isolated wetland elevations range from 34 m in the Upper Coastal Plain to 104 m in the Sandhills region.

There are approximately 300 isolated wetlands on the SRS; they are at lowest densities in the northwestern portion of the site and occur in clusters of 2–14 wetlands (Schalles et al. 1989). From the known isolated wetlands on the SRS, we randomly selected several wetlands within each cluster for sampling. This ensured that representative isolated wetlands were sampled across the entire landscape of the study area (Fig. 1).

Ecological studies of isolated wetlands at the SRS have been of Carolina bays. Soils of SRS Carolina bays are mineral with little or no peat deposits (Frey 1950; Schalles and Shure 1989). Water chemistry is primarily driven by precipitation and exhibits a dystrophic condition, although groundwater influences are evident under some hydrological conditions (Schalles 1989). In general, the annual hydrological pattern of isolated wetlands is seasonal with highest water levels occurring during winter months and lowest levels during late summer months. This seasonal pattern corresponds to low and high periods of transpiration. The hydrology of individual bays is influenced by local precipitation patterns, the permeability of underlying strata, and local groundwater conditions (Schalles and Shure 1989; Lide et al. 1995). Drying frequency ranges from annually to only during severe drought and usually corresponds with the late summer low water period. Little is known of the timing of connections to permanent habitats, but presumably these connections occur during the winter high water period. Primary production in Carolina bays is low to moderate (Tilly 1973; Schalles and Shure 1989) and secondary production is relatively high in comparison with surrounding terrestrial habitats and nearby, more permanent aquatic habitats (Congdon and Gibbons 1989; Taylor et al. 1989).

Sampling

Isolated wetlands were visited during the summer of 1994. If the wetland contained water it was sampled for fishes and physical variables. Fishes were sampled with baited minnow and hoop net traps. Minnow traps had ~3-cm openings and 6-mm square mesh. Hoop traps were constructed of hoops that were 70 cm in diameter and 0.16-cm square mesh netting. Traps were baited with commercial catfish bait and biscuit dough.

All habitat types within a wetland were trapped for at least 2 trap days (1 trap day = 1 trap fished for 24 h) before a wetland was declared not to be occupied by fishes. The minimum amount of effort that resulted in a classification of no fishes was 4 trap days in a small, relatively simple wetland. Wetlands with fish populations were trapped until a species accumulation curve for that wetland became asymptotic.

Temperature, pH, dissolved oxygen, total dissolved solids, specific conductance, and redox potential were measured using a Hydro-lab at one to several locations in each wetland that had standing water. In diverse wetlands with several habitat types (e.g., emergent marsh, floating marsh), measurements were made at one point in each habitat type. If depth allowed, measurements were made at the surface (~2.0 cm below the surface), at middepth, and on the bottom (~5 cm from the bottom) at each point sampled. In shallow conditions measurements were made at the surface and bottom or middepth only. A maximum water depth for each wetland was also determined. The majority (~90%) of the above physical measurements were made on 2 consecutive days, August 8 and 9, 1994, to minimize any seasonal variation between locations.

The area of each wetland was calculated from aerial photography using ERDAS software (MEASURE module). The majority of wetlands included in our survey were visited five times during 1990 and the presence or absence of water was noted. A relative measure of the frequency of drying was calculated as the number of times the wetland was visited that it held water. Our score ranged from 5 for wetlands that seldom dried to 1 for wetlands that frequently dried.

Landscape position

We used the center of each wetland as a reference point for distance measurements, rather than the edge, because of the ambiguity that arises in defining the edge of a wetland; pines may become established around the edges of wetlands at the SRS during dry periods, but they are killed during periods of high water levels. The center point of each wetland was plotted on 1 : 24 000 scale U.S. Geological Survey (USGS) quadrangle maps using universal transverse Mercator (UTM) grids. We measured the shortest distance to the nearest permanent and intermittent aquatic habitats. We recognized two types of permanent aquatic habitats (PAHs): streams and reservoirs. We further divided streams into headwater and channel types. If the nearest PAH was the terminus of a permanent stream we classified the PAH as a stream head. If it was a point on a stream between its terminus and its confluence with another stream, the PAH was classified as a stream channel. We recognized three intermittent aquatic habitats (IAHs): intermittent streams, other isolated wetlands, and riverine swamps. Riverine swamps are located along the Savannah River in our study area and differed from isolated wetlands in not being isolated topographically, but rather exhibiting slow drainage to the Savannah River because of a low gradient.

The distance between two isolated wetlands was measured from

the center points on the maps. Swamps were identified on CIR aerial photography (January 1992, nominal scale of 1 : 15 840). Distance was measured from the center point of the isolated wetland to the swamp edge. The centers of isolated wetlands were visually located on the photographs and the edge of the swamp was identified as the transition from bottomland hardwood forest to upland pine plantation. The elevations of isolated wetlands and their nearest PAHs were defined as the midpoint elevation between contour lines within which wetlands and PAHs were found on the quadrangle maps (3-m intervals).

Analysis

We expressed fish densities as catch per unit effort (CPUE). We did not distinguish between trap types because traps were fished as pairs; thus, the total amount of effort may have differed between wetlands, but the relative use of the two trap types did not. Fish assemblages were clustered on the basis of Pearson correlation coefficients.

We used Monte Carlo methods, following Connor and Simberloff (1979) and Matthews (1982), to test the hypothesis that random processes are sufficient to explain differences in assemblage structure between wetlands. Briefly, we tested the null hypothesis that variation in the number of wetlands in which a species occurred could be explained by chance alone by randomly placing species into individual wetlands constrained by the total number of species found to occur in each wetland. Ten matrices were produced in this way to generate a distribution for comparison with the observed distribution of species. We tested the hypothesis that the number of wetlands shared by species pairs could be explained by chance alone by further random rearrangement of the 10 matrices generated earlier. Matrices were rearranged so that both the number of species in individual wetlands (row totals) and the number of wetlands in which a species occurred (column totals) were constrained.

We tested the null hypothesis that species distributions between wetlands were no more nested than would be expected under laws of probability following Patterson and Atmar (1986). In a perfectly nested structure, the species occurring in one wetland would be a subset of the species occurring in the next richest wetland, so that the richest wetland has all of the species found in all of the more species-poor wetlands. We calculated an index (N) for the 10 random matrices and our observed matrix of fish distribution. For the random matices we calculated N after rearrangement to constrain for the number of wetlands in which a species was found because this is the most restrictive of the two models presented by Patterson and Atmar (1986). N was calculated by ordering the assemblages from the most species rich to the most species poor. For each species the most species-poor assemblage in which the species occurred was identified and N_i was calculated as the number of more species-rich assemblages in which the species was not found. N_i values were summed to get N. Student's t tests were used to compare observed and expected values generated from random expectations. The overall error rate for these comparisons was controlled at $p = 0.05$ using the Bonferroni method. Chi-square tests were used to compare the observed and expected distributions of species between wetlands and the number of wetlands shared by species pairs.

For analytical purposes we divided the physical variables into two groups: environmental (reflecting the internal environment of wetlands) and landscape (reflecting the relative position of wetlands). Log(x + 1) transformations were used to transform all or subsets of the variables in each group when variances or normality assumptions were violated.

We removed variation in dissolved oxygen, temperature, and redox potential associated with time of day as follows: we used analysis of variance (ANOVA) to test for a significant effect of time of day by placing measurements into morning (9:00–11:00), midday (11:00–13:00), and afternoon (13:00–15:00) groups. If a significant difference was found a simple linear regression model (with the environmental variable as the dependent variable and time of day as the independent variable) was fitted to the data. Because all measures were made between 9:00 and 15:00 a linear model provided the best fit. Individual measurements were then standardized to 12:00 (noon) by subtracting the difference between the predicted value and a line passing through 12:00 with zero slope from the observed value. All statistical analyses were performed on the transformed and standardized data sets.

Principal components analysis (PCA) was used to summarize variance in the environmental and landscape variables. Our goal in these analyses was to determine if a weighted set of variables was a better predictor of assemblage structure than the individual variables considered separately. Averages of all measures of temperature, pH, dissolved oxygen, redox potential, specific conductance, and total dissolved solids for each wetland sampled were entered into PCA. Because we did not have drying scores for all wetlands having water, we used the pairwise deletion PCA procedure of SYSTAT (Wilkinson 1987) for PCA of environmental variables. This procedure calculated correlations separately for each pair of variables and avoided the deletion of entire cases because of missing data for a single variable. Environmental PCA scores were only calculated for those wetlands with complete sets of data.

We used t tests to compare the mean values of continuous environmental and landscape variables, and PCA scores, between wetlands with and without fish assemblages. PCA scores were found to be normally distributed before t tests were conducted. Pearson correlation coefficients were used to investigate the relationship between environmental and landscape variables, and PCA scores, and total abundance of fishes and abundance of individual species. Mean environmental and landscape variables and PCA scores for fish assemblage clusters were compared using ANOVA. Tukey's W procedure was used to compare means.

Results

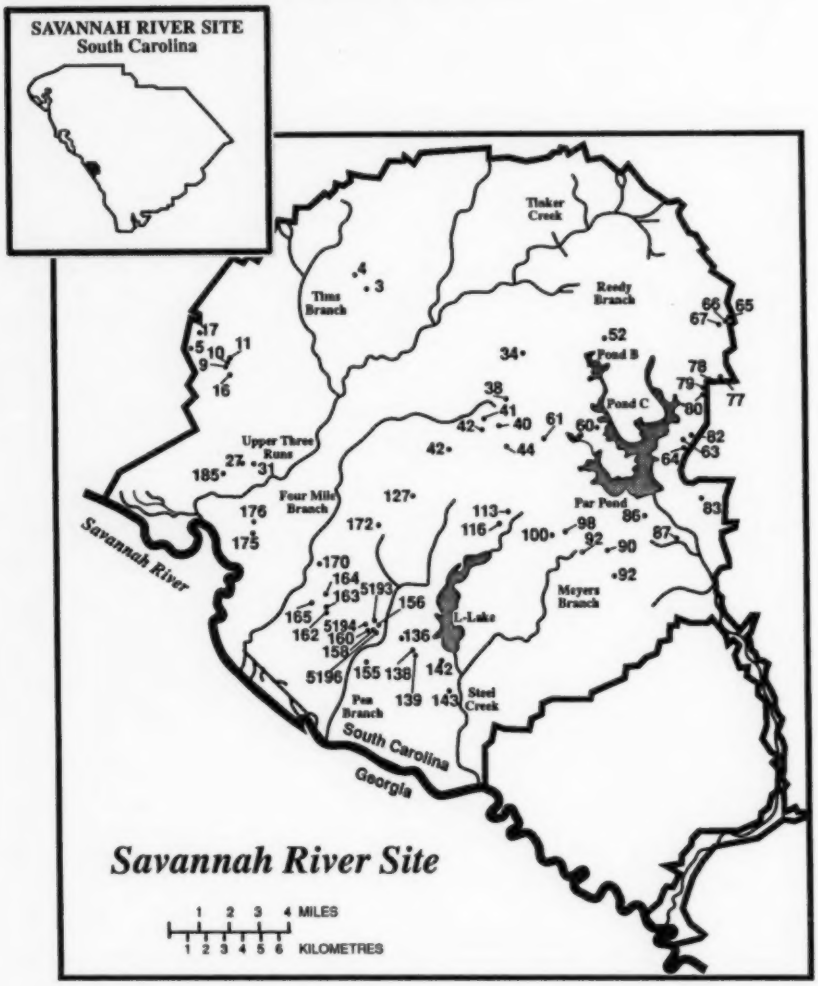
Fishes

We visited 63 isolated wetlands (Fig. 1) of which 29 held water and were sampled for fishes and environmental variables. Thirteen of the wetlands contained fish populations. The richness of wetland fish assemblages ranged from one to six species (Table 1). Although the CPUE was near 4 fish/day for each trap for most wetlands, it ranged from 0.04 fish/day for each trap in wetland 5193 to 23.21 fish/day for each trap in wetland 78. Number of species and CPUE were positively correlated ($r = 0.57$, $p = 0.04$).

On the basis of the number of wetlands in which a species occurred, two groups of species can be recognized: widespread species, occurring in seven or more wetlands (e.g., *Erimyzon sucetta*), and restricted species, occurring in four or fewer wetlands (e.g., *Ameiurus natalis*; Table 2). This dichotomy in species' distributions among wetlands was evident in our Monte Carlo analysis. There were more species occurring in one, seven, eight, and nine wetlands than would be expected by chance (Fig. 2A). Alternatively, there were fewer species occurring in four and five wetlands than would be expected by chance. However, these differences between the observed and expected distributions were only marginally significant ($\chi^2 = 13.7$, $p = 0.09$).

The distribution of fishes among wetlands was significantly nested (observed $N = 34$, expected $N = 51.2$, $p < 0.01$). In other words, restricted species were found in the more diverse wetland assemblages and species-poor assemblages consisted of widespread species only. The number of mutually exclusive pairs of species was not different from that expected under a

Fig. 1. Location of the Savannah River Site in South Carolina, and locations and identification numbers of wetlands visited during the summer of 1994.



model of random distribution of species among wetlands ($\chi^2 = 3.6$, $p = 0.57$, Fig. 2B).

Cluster analysis identified four groups of wetlands with similar fish assemblages (Fig. 3). These clusters reflect differences among wetlands in the abundance of the three widespread species, *Acantharchus pomotis*, *Lepomis marginatus*, and *E. sucetta*, and the restricted species, *Gambusia holbrooki*. For example, all wetlands in the *Acantharchus pomotis* cluster have larger populations of *Acantharchus pomotis* than other widespread species or *G. holbrooki* (Table 2). While the dominant species in most wetlands was a widespread species or *G. holbrooki*, there were exceptions. *Umbra pygmaea*, *Centrarchus macropterus*, and *Notemigonus crysoleucas*, all restricted species (Table 2), were the dominant species in one wetland each (Table 1).

Wetland environments

Although some of the environmental variables showed patterns with depth there was much variation among wetlands (Fig. 4). Among environmental variables, four ecologically interpretable principal components accounted for 81.7% of the variance (Table 3). We interpret PC I as a productivity gradient from relatively unproductive wetlands with low specific conductance, pH, and total dissolved solids and high redox potential to more productive wetlands with high specific conductance, pH, and total dissolved solids and low redox potential. PC II reflects the influence of maximum water depth on dissolved oxygen, and it is a gradient from relatively deep wetlands with low average dissolved oxygen levels to more shallow wetlands with higher average dissolved oxygen levels. PC III is a temperature gradient. We interpret PC IV as reflecting the effects of wetland size on drying frequency. This component is a gradient from large wetlands that dry frequently to small wetlands that dry less frequently.

Landscape position

Among landscape variables, four principal components

Table 1. Density of fish species (as catch per unit effort, no. of fish caught per day in each trap) and richness in isolated wetlands at the SRS during the summer of 1994.

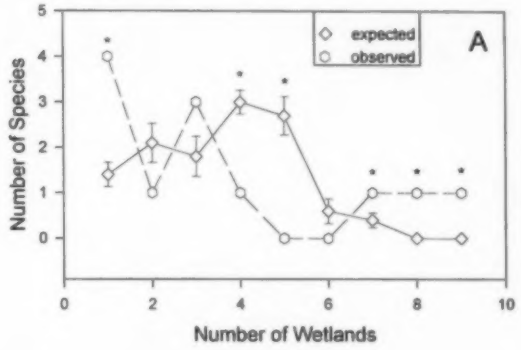
	Wetland No.												
	138	5193	143	86	40	78	42	77	41	97	100	142	136
Erimyzon sucetta	0.00	0.00	0.00	0.13	0.27	0.50	0.62	0.04	0.58	5.94	3.64	1.11	0.00
Lepomis marginatus	0.04	0.00	0.00	0.00	0.42	21.57	3.21	0.20	0.10	0.02	0.00	0.00	0.00
Gambusia holbrooki	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.90	4.22
Acantharchus pomotis	0.63	0.04	1.44	0.38	0.09	0.50	0.00	0.00	0.00	0.02	0.00	0.07	0.00
Centrarchus macropterus	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00
Lepomis gulosus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.41	0.00	0.07	0.00
Notemigonus crysoleucas	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.92	0.00	0.00	0.14	0.00
Umbra pygmaea	0.00	0.00	1.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Esox americanus	0.00	0.00	0.00	0.00	0.00	0.21	0.04	0.00	0.05	0.06	0.00	0.00	0.00
Ameiurus natalus	0.00	0.00	0.00	0.00	0.00	0.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ameiurus nebulosus	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00
Enneachanthus gloriosus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00
Total	0.67	0.04	3.13	1.49	0.80	23.21	4.08	0.56	1.64	6.74	3.64	3.36	4.22
Richness	2	1	3	3	4	5	4	4	4	5	1	6	1

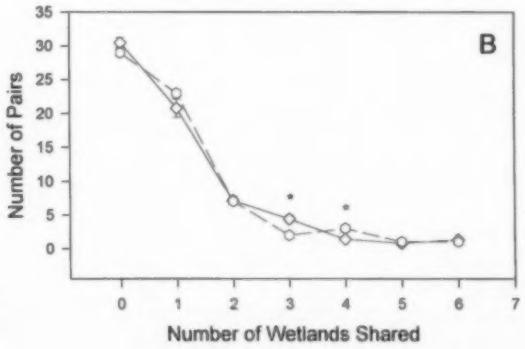
Table 2. Absolute and relative abundance and distribution of fishes in and among isolated wetlands of south-central South Carolina during the summer of 1994.

	Abundance		Wetlands occupied	
	No.	%	No.	%
Erimyzon sucetta	474	38	9	69
Lepomis marginatus	418	33	7	54
Gambusia holbrooki	165	13	3	23
Acantharchus pomotis	59	5	8	62
Centrarchus macropterus	36	3	2	15
Lepomis gulosus	25	2	3	23
Notemigonus crysoleucas	24	2	3	23
Umbra pygmaea	19	1	1	08
Esox americanus	8	1	4	31
Ameiurus natalus	6	<1	1	8
Ameiurus nebulosus	6	<1	1	8
Enneacanthus gloriosus	1	<1	1	8
Total	1254			

Note: Relative abundance is the percentage of the total number of fish collected represented by species *x*; relative distribution is the percentage of wetlands having fish populations in which the species occurred.

Fig. 2. Distributions of the number of species versus the number of wetlands in which they occurred (A), and the number of species pairs versus the number of wetlands shared (B). Expected values are based on Monte Carlo simulations following Conner and Simberloff (1979) and Matthews (1982). Asterisks indicate significant differences ($p < 0.05$) between observed and expected values at given levels of number of wetlands occupied by a species (A), and number of wetlands shared by a pair of species (B).





accounted for 88.5% of the variance (Table 4). PC I reflects the position of wetlands in the study area and is a continuum from wetlands with high elevations located in the northeastern portion of the study area to wetlands with low elevations in the southwestern portion. The elevation of the nearest PAH was also correlated with PC I, reflecting an overall decrease in this variable from the northeastern to the southwestern portion of the study area. The type of the nearest IAH was also correlated with PC I, reflecting a shift from intermittent streams in the upland portions of the study area to swamps in the lowland portions along the Savannah River. PC II is a connectivity continuum from wetlands close to IAH and PAH with little elevation difference between the wetland and the nearest PAH to wetlands more removed from IAH and PAH with a greater

Fig. 3. Results of cluster analysis of fish assemblages based on Pearson correlation coefficients. Recognized clusters reflect the dominant species in the assemblage. Numbers are wetland identification numbers from Fig. 1.

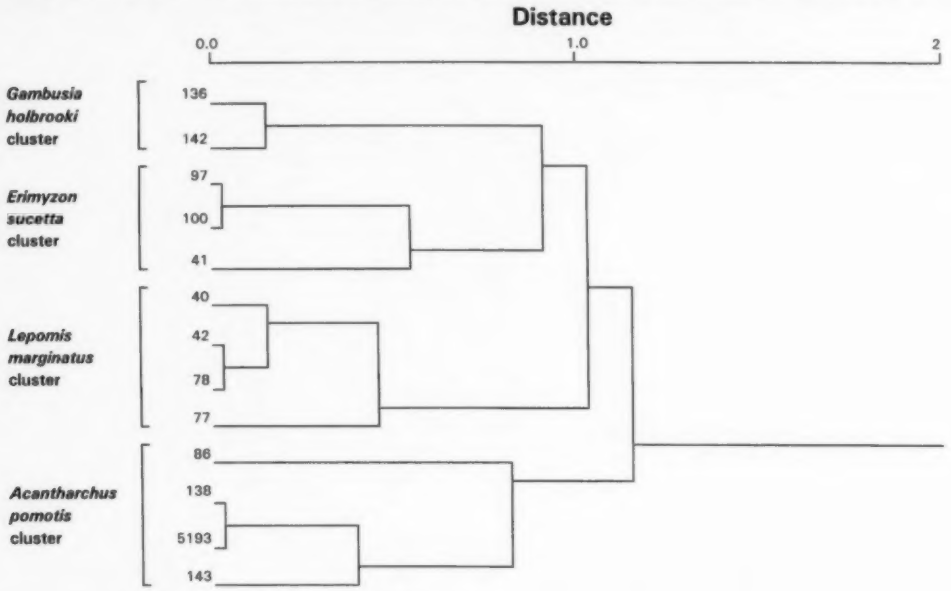


Fig. 4. Means and ranges (horizontal bars) of physical variables along a depth gradient in isolated wetlands of the southeast.

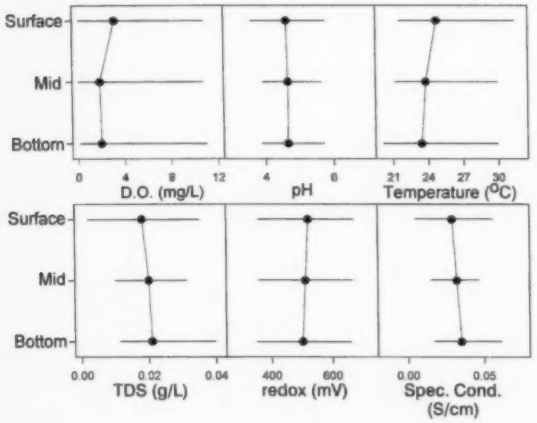


Table 3. Results of principal components analysis of environmental variables measured in isolated wetlands of south-central South Carolina.

	PC I	PC II	PC III	PC IV
Drying score	—	0.462	—	0.760
Total dissolved solids	0.866	—	—	—
Specific conductance	0.840	0.312	—	—
Log wetland size	—	−0.476	—	−0.692
Log maximum water depth	—	−0.826	—	—
Log pH	0.763	—	0.318	—
Log dissolved oxygen	—	0.817	0.445	—
Log temperature	0.323	—	0.897	—
Redox potential	−0.745	—	—	—
% of variance explained	30.8	22.4	15.0	14.3

Note: Loadings of variables on the first four principal components and the percentage of variance explained by each component is given. For clarity only loadings >0.300 are listed. Log dissolved oxygen, log temperature, and redox potential were first corrected for time of day of measurements (see Methods).

elevation difference between the wetland and PAH. PC III reflects the correlation between IAH type and distance to the nearest IAH. When swamps were the nearest IAH, the distance between the swamp and the isolated wetland tended to be shorter than when the nearest IAH was an intermittent stream or another isolated wetland. PC IV reflects additional variation in the type of the nearest PAH and its correlation with other landscape variables. In general, PC IV reflects a shift from wetlands located near stream heads in the western portion of the SRS to wetlands located near stream channels and reservoirs in the eastern portion. No landscape variables were strongly correlated with PC IV (Table 4), and thus we do not consider this principal component further.

Relationship of fishes to physical habitat and landscape position

Wetlands with fish populations tended to cluster relative to environmental PC II and III (Fig. 5). No wetlands with fishes had high scores on environmental PC II, suggesting that shallow water, but not dissolved oxygen, may be a limiting factor for fishes. Similarly, wetlands with fishes did not have low scores on PC III, suggesting that fishes are limited to wetlands with higher temperatures. Despite these apparent patterns

there were no significant differences in mean scores along the four PC axes for wetlands with and without fishes. When the means of the environmental variables are considered individually, only mean drying scores differed between wetlands with and without fishes ($p < 0.01$); wetlands with fishes dried less frequently than wetlands without fishes. The mean size of wetlands with fishes was marginally greater than that of wetlands without fishes ($p = 0.09$).

Isolated wetlands were less likely to have fishes than more connected wetlands (Fig. 6); however, the differences were only marginal ($p = 0.08$). This was more true for wetlands in the low elevation southwestern portion of the SRS than for wetlands in the higher elevation northeastern portion of the SRS. There was no relation between the presence and absence of fishes and landscape PC III or IV (Fig. 6). When the landscape variables are considered individually, only mean distance to the nearest IAH was different ($p = 0.01$) between wetlands with and without fishes. Mean distance to the nearest IAH was less for wetlands with fishes.

We further analyzed the relationship between drying score and distance to IAH using direct gradient analysis (Gauch 1982; Fig. 7A). This analysis defines a subset of wetlands in the lower right corner of Fig. 7A that may have fish populations. Because of the number of wetlands falling in this region, we tested for significant differences between mean environmental and landscape variables between wetlands with and without fish among this subset. Only elevation difference between the wetland and the nearest PAH was significant ($p = 0.05$). While elevation difference further discriminated wetlands with and without fishes (Fig. 7B), a number of wetlands without fishes had low elevation differences and one wetland with fishes (No. 78) had a large elevation difference. We were unable to detect further differences in environmental or landscape variables between wetlands falling in the bottom right corner of Fig. 7B.

Species richness was positively correlated with temperature and environmental PC III scores (Table 5). Richness was not correlated with any of the landscape variables or PC scores. Fish density was not correlated with any of the environmental or landscape variables or PC scores. Overall there was little correlation between environmental or landscape variables and PC scores, and the abundance of individual species (Table 5).

Although the landscape positions of wetlands occupied by the four different fish assemblage types identified by cluster analysis (Fig. 8) differed, their environments did not ($p > 0.10$). The *E. sucetta* and *L. marginatus* groups had higher mean wetland elevations, higher elevations of the nearest PAH, and higher PC I scores than the *G. holbrooki* and *Acantharchus pomotis* groups ($p < 0.01$). In addition, the mean northerly UTM coordinate for the *L. marginatus* group was greater than for the *E. sucetta* and *G. holbrooki* groups ($p < 0.01$).

All wetlands with fishes were nearest to stream heads with the exception of wetland 86, which was nearest to a stream channel. There was more variability in the type of the nearest IAH among the assemblages. All of the *L. marginatus* and *E. sucetta* assemblages were nearest to other isolated wetlands, with the exception of wetlands 40 and 42, which were closest to intermittent streams. Of the *Acantharchus pomotis* and *G. holbrooki* assemblages, three were nearest to intermittent streams, one was nearest to another isolated wetland, and two were nearest to swamps. We did not test for the independence of nearest aquatic habitat types and assemblage types because of low cell counts.

Table 4. Results of principal components analysis of landscape position variables for isolated wetlands in south-central South Carolina.

	PC I	PC II	PC III	PC IV
Log distance to permanent habitat	—	0.903	—	—
Type of permanent habitat	0.635	—	—	–0.550
Log of wetland elevation	0.931	—	—	0.306
Log of permanent habitat elevation	0.890	—	—	0.316
Log elevation difference	—	0.924	—	—
Log distance to intermittent habitat	—	0.647	–0.577	—
Type of intermittent habitat	–0.408	0.390	0.691	0.324
Log easterly UTM coordinate	0.747	—	0.367	–0.408
Log northerly UTM coordinate	0.708	0.368	—	0.398
% variance explained	37.3	28.0	12.2	11.0

Note: Loadings of variables on the first four principle components and the percentage of variance explained by each component is given. For clarity only loadings >0.300 are listed.

Geographically, there was a shift from assemblages dominated by *L. marginatus* or *E. sucetta* (in the north and northeast) to those dominated by *G. holbrooki* or *Acantharchus pomotis* (in the southwest) on the SRS (Fig. 8A). Along this same geographic gradient there is a shift from true headwater streams to tributary streams (streams that connect to a main channel in the downstream portion of the drainage), which we did not include in our original analysis. As a measure of this gradient we calculated the downstream link (Osborne and Wiley 1992) of the nearest permanent stream to each of the wetlands with fishes. The downstream link of a stream is the number of first-order streams upstream of the confluence of the stream with the main channel of the drainage basin (Osborne and Wiley 1992). The fish assemblages clearly separate into headwater (*L. marginatus* and *E. sucetta* groups) and downstream (*G. holbrooki* and *Acantharchus pomotis* groups) groups along this gradient (Fig. 8B).

Discussion

We found fishes in 21% of the wetlands we sampled, which is higher ($z = 42.3$, $p < 0.001$) than the 10% suggested for Carolina bays (Sharitz and Gibbons 1982; Richardson and Gibbons 1993), and at the upper end of the range of 10–20% reported for wetlands in the prairie region of Manitoba (Lawler et al. 1974). There are two possible reasons for our higher estimate. First, not all of the isolated wetlands we sampled would meet the geological and geomorphological criteria of Carolina bays. Second, the number of isolated wetlands with fishes may be highest following several years of above-average rainfall during which successful colonization events are common and complete drying, and thus extirpation events, are rare. An extended period of low rainfall has not occurred since June 1990 (SRTC Weather Center, Aiken, S.C.).

Fig. 5. Plots of wetlands with and without fishes in environmental principal component space. The positions of some wetlands are not plotted because of incomplete data sets.

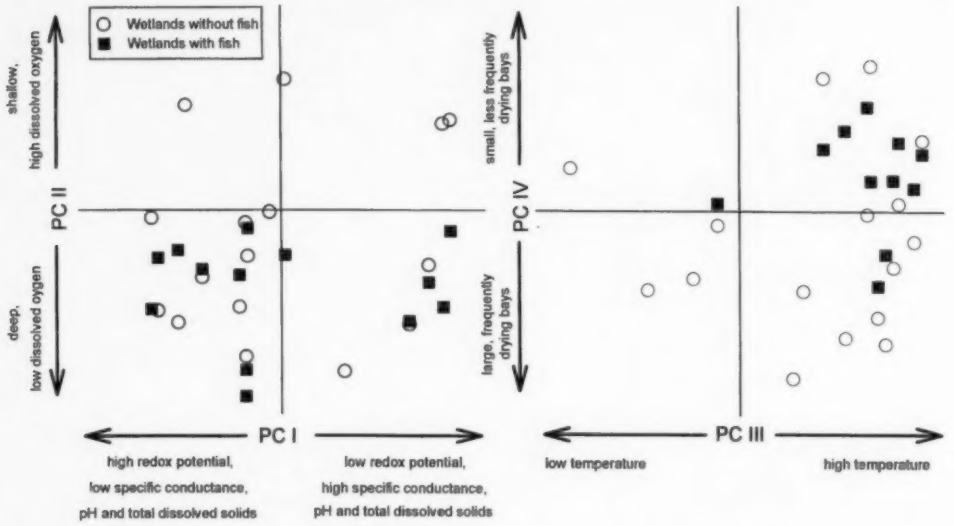
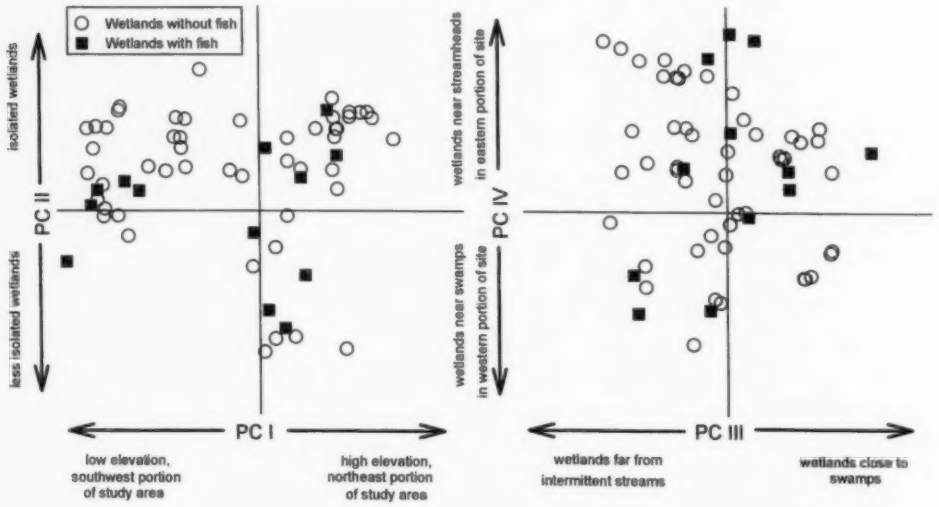

Wetlands without fish
Wetlands with fish
shallow, high dissolved oxygen
deep, low dissolved oxygen
PC II
small, less frequently drying bays
large, frequently drying bays
PC IV
PC I
high redox potential, low specific conductance, pH and total dissolved solids
low redox potential, high specific conductance, pH and total dissolved solids
PC III
low temperature
high temperature

Fig. 6. Plots of wetlands with and without fishes in landscape principal component space.


Wetlands without fish
Wetlands with fish
isolated wetlands
less isolated wetlands
PC II
wetlands near streamheads in eastern portion of site
wetlands near swamps in western portion of site
PC IV
PC I
low elevation, southwest portion of study area
high elevation, northeast portion of study area
PC III
wetlands far from intermittent streams
wetlands close to swamps

At the coarse scale of presence or absence of fishes, we hypothesized that drying frequency (disturbance frequency) and nearness to a source of colonists would be important. This hypothesis was supported; wetlands with fishes dried less frequently and were more connected (closer to IAHs) than wetlands without fish. However, we were unable to detect differences in landscape position or environments between a number of wetlands with and without fishes. Similarly, Rahel (1984) was unable to detect differences in the environments of bog lakes with and without fishes. It is possible that fishes are occasionally extirpated from bog lakes (because of low winter oxygen levels) as they are from isolated wetlands (because of drying). In this situation, one would expect to find some lakes or wetlands that have not received colonists since last disturbed because of local variation in surface water hydrology.

We did find structure in the wetland fish assemblage beyond that expected from random chance. Species were either underdistributed (restricted) or overdistributed (widespread), and species-rich assemblages were made up of restricted and widespread species while species-poor assemblages were made up of widespread species only (species were significantly nested). This structure, along with the lack of structure associated with mutually exclusive species pairs (Fig. 2B), suggests that competition and predation are not strong structuring

mechanisms in these wetlands. This is consistent with the idea that in frequently disturbed communities, biotic interactions will not be strong structuring forces (Menge and Sutherland 1976; Wiens 1984; Poff and Ward 1989).

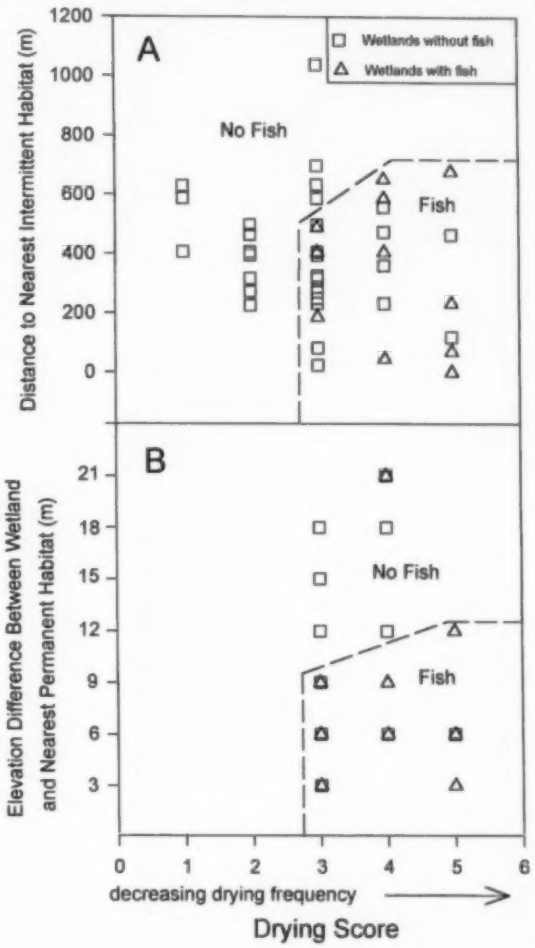
The lack of correlation between environmental variables and the abundance of individual species suggests that differences in the environment between wetlands (other than drying frequency) are not responsible for structuring wetland fish assemblages. Thus, we suggest that the interaction of colonization and extinction processes and variation in the frequency and duration of connections between wetlands and permanent aquatic habitats are responsible for the nested structure of wetland fish assemblages. Under this scenario, species richness will be highest in wetlands that are connected often and for longer periods because more populations will be rescued from extinction by frequent immigration events (sensu Brown and Kodric-Brown 1977), and poorly colonizing species will have a greater chance of reaching these wetlands.

Characteristics of the connections between wetlands and permanent habitats may also contribute to the relation of fish assemblage structure and landscape position by acting as a screen on the pool of possible colonists. For example, *G. holbrooki* was limited to wetlands in the lower portion of the study area (Table 1, Fig. 1). In this area, a gradual rising of water in both proximate riverine swamps and wetlands will establish connections, and these connections are likely to have little or no current. In contrast, wetlands in the upper portion of the study area overflow to intermittent streams, and thus have swift currents that may prevent small fishes such as *G. holbrooki* from using the connection as a colonization route.

Consideration of wetland 78, which had fish but also had a large elevation difference between the wetland and the nearest PAH, also suggests the importance of connection properties. Association of this wetland with the second nearest PAH (in a different drainage) considerably reduces the elevation difference between the wetland and PAH (from ~18 to ~3 m). Thus, it is possible that colonists of this wetland do not come from the nearest PAH, but from the most connected PAH. This may be why isolated wetlands in the southwestern portion of the site are less likely to have fishes (Fig. 6). Elevation relief is not great in the southwestern portion of the site, and thus distance to the nearest IAH and elevation difference both determine the connectedness of wetlands. In the northeastern portion of the site, elevation relief is greater, and thus elevation difference becomes the overriding factor in determining the connectedness of wetlands.

While landscape position was correlated with the abundance of widespread species, a lack of correlation between species richness and landscape position suggests that other factors may also be important in controlling the frequency and duration of connections, and thus colonization rates. The most likely mechanism is local variation in surface water levels associated with local variation in rainfall or internal properties of the wetland (e.g., groundwater connections). If water level variations are driven by variation in rainfall across the landscape, then surface water level variation will be unpredictable from year to year, and fish assemblage structure will be variable. Alternatively, if variation is driven by specific characteristics of each wetland then surface water level variation will be irrelevant to the biota, and assemblage structure will be relatively stable.

Fig. 7. Direct gradient analysis of drying score, distance to nearest intermittent habitat (A), and elevation difference between wetlands and their nearest permanent habitats (B). Broken lines indicate the area of the graph in which wetlands with fish fall and were drawn by hand.



These findings are inconsistent with studies of other lentic environments. The distribution of cyprinids among Wisconsin (U.S.) lakes is limited by competition or predation from centrarchids, which are limited by severe environmental conditions, primarily low winter dissolved oxygen levels (Tonn and Magnuson 1982; Rahel 1984). The distribution of fishes in prairie basin wetlands is a function of low winter oxygen levels, although drying frequency is also important (Peterka 1989). The difference between these northern systems and the wetlands we studied was the lack of the development of anoxic conditions during summer or winter in our area. While the edges of wetlands at the SRS may have ice during winter months, the entire surface seldom if ever freezes (Schalles et al. 1989). Although low oxygen conditions develop in

Table 5. Matrix of Pearson correlation coefficients between assemblage properties and abundance of individual species, and environmental variables and wetland scores based on derived principal components.

	Drying score	Total dissolved solids	Specific conductance	Log size	Log maximum water depth	Log pH	Log dissolved oxygen	Log temperature	Log redox potential	PCA Score I	PCA Score II	PCA Score III	PCA Score IV
Richness	–0.03	–0.42	–0.39	0.34	–0.13	0.06	–0.15	0.67*	0.00	–0.12	–0.06	0.63*	–0.36
Total fish	–0.08	–0.58	–0.58	–0.02	–0.34	–0.21	0.14	0.21	0.18	–0.36	0.17	0.26	–0.10
L. marginatus	0.02	–0.49	–0.49	–0.07	–0.38	–0.16	0.16	0.29	0.13	–0.28	0.22	0.32	–0.01
E. sucetta	–0.10	–0.26	–0.24	0.37	0.26	–0.06	–0.05	–0.17	0.10	–0.19	–0.29	–0.04	–0.24
G. holbrooki	–0.26	0.15	0.15	–0.37	0.29	0.06	–0.27	–0.18	–0.12	0.12	–0.06	–0.34	0.07
A. pomotis	–0.03	–0.16	–0.20	0.01	–0.59*	–0.51	0.02	–0.24	0.39	–0.38	0.39	–0.17	0.04
N. crysoleucas	0.03	0.11	0.11	–0.44	0.01	0.57	0.05	0.52	–0.60*	0.44	0.03	0.30	0.03
L. gulosus	–0.49	–0.42	–0.39	0.61*	0.13	–0.30	0.12	0.07	0.37	–0.41	–0.06	0.18	–0.62
E. americanus	–0.01	–0.56	–0.56	–0.05	–0.33	–0.11	0.23	0.41	0.09	–0.28	0.22	0.42	–0.14

Note: Values used in the correlation of total fish and individual species were catch per unit effort (fish caught per day in each trap). Log dissolved oxygen, log temperature, and log redox potential were corrected for time of day of measurements before being entered into principal components analysis. See text for methods of correction.

* Significant at $p < 0.05$.

Fig. 8. Plot of the geographical locations of the different assemblage types identified by cluster analysis (A), and location of wetlands with fishes along a downstream link gradient (B).

portions of isolated wetlands at the SRS during summer months, completely anoxic conditions normally do not occur (Schalles 1989), possibly because of the low to moderate primary productivity of these systems (Schalles and Shure 1989).

The relative locations of lentic as well as lotic habitats influence fish assemblage structure. In Wisconsin lakes with low winter oxygen levels, lakes connected to a stream had different assemblages than lakes isolated from streams (Tonn and Magnuson 1982). In lotic systems, first- and second-order streams in the headwaters of a drainage had fewer species than first-or second-order streams located in the lower portions of the drainage (Osborne and Wiley 1992). Our results suggest that the relative abundance of species in the nearest source pool of colonists, first-order streams in most cases, differs between headwaters and downstream portions of drainages at the SRS. Osborne and Wiley (1992) argued that increased richness with downstream drainage position is a result of increased colonization rates and decreased extinction rates resulting from a proximate source of colonists in larger stream channels. If patterns of wetland fish assemblage structure reflect patterns of source assemblage structure, then our results suggest that source populations in downstream channels may affect the relative abundance of species, as well as species richness, in proximate low-order streams, possibly through source–sink population interactions (Pulliam and Danielson 1991).

Fish species richness in lentic and lotic habitats has been

associated with some measure of size in many studies (e.g., Larimore and Smith 1963; Sheldon 1968; Barbour and Brown 1974; Browne 1981; Angermeier and Schlosser 1989). This relationship has been attributed to habitat complexity (Tonn and Magnuson 1982), differential immigration and extirpation rates (Power et al. 1988), and sampling phenomena (Angermeier and Schlosser 1989). We found no correlation between wetland size and fish species richness among wetlands having fishes in our study area. We view this as a result of the interaction between disturbance frequency and the relative importance of colonization rates. In frequently and severely disturbed systems, assemblage structure will depend heavily on colonization rates. In more benign systems, biotic interactions will be more important in structuring assemblages, and habitat complexity, and thus size, becomes important. We suggest that the wetlands we studied lie at the frequently and severely disturbed end of this continuum, and thus colonization rate is important in determining assemblage structure, and size is not.

In conclusion, our data support a two-level model of fish assemblage structure in isolated wetlands of the Upper Atlantic Coastal Plain. At a coarse level, the presence or absence of fishes is determined by the frequency of drying and the connectivity of wetlands to a permanent source of colonists. At the scale of variation in assemblage structure between wetlands with fish populations, differences are a function of differences in the relative abundance of species in colonist source pools and the amount of time wetlands have been connected to more permanent habitats since they last dried. Broader geographical surveys and information on the identity of species reaching wetlands during periods of colonization and temporal variation in assemblage structure are needed to validate this model.

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References

Angermeier, P.L., and Schlosser, I.J. 1989. Species-area relationship for stream fishes. Ecology, **70**: 1450–1462.

Barbour, C.D., and Brown, J.H. 1974. Fish species diversity in lakes. Am. Nat. **108**: 473–489.

Bennett, D.H., and McFarlane, R.W. 1983. The fishes of the Savannah River Plant: National Environmental Research Park. National Environmental Research Park publication. U.S. Department of Energy and Savannah River Ecology Laboratory, Aiken, S.C.

Brown, J.H., and Kodric-Brown, A. 1977. Turnover rates in insular biogeography: effects of immigration on extinction. Ecology, **58**: 445–449.

Browne, R.A. 1981. Lakes as islands: biogeographic distribution, turnover rates, and species composition in the lakes of central New York. J. Biogeogr. **8**: 75–83.

Congdon, J.D., and Gibbons, J.W. 1989. Biomass productivity of turtles in freshwater wetlands: a geographical comparison. *In* Freshwater wetlands and wildlife. U.S. Department of Energy Conference Series No. CONF-8603101. *Edited by* R.R. Sharitz and J.W. Gibbons. U.S. Department of Energy, Washington, D.C. pp. 583–592.

Connor, E.F., and Simberloff, D. 1979. The assembly of species communities: chance or competition? Ecology, **60**: 1132–1140.

Frey, D.G. 1950. Carolina bays in relation to the North Carolina coastal plain. J. Elisha Mitchell Sci. Soc. **66**: 44–52.

Frey, D.G. 1951. The fishes of North Carolina's bay lakes and their intraspecific variation. J. Elisha Mitchell Sci. Soc. **67**: 1–44.

Gauch, H.G., Jr. 1982. Multivariate analysis in community ecology. Cambridge Stud. Ecol. Vol. 1.

Larimore, R.W., and Smith, P.W. 1963. The fishes of Champaign County, Illinois as affected by 60 years of stream change. Ill. Nat. Hist. Surv. Bull. **28**: 299–382.

Lawler, G.H., Sunde, L.A., and Whitaker, J. 1974. Trout production in prairie ponds. J. Fish. Res. Board Can. **31**: 929–936.

Lide, R.F., Meentemeyer, V.G., Pinder, J.E., III, and Beatty, L.M. 1995. The hydrology of a Carolina bay located on the Upper Coastal Plain of western South Carolina. Wetlands, **15**: 47–57.

Matthews, W.J. 1982. Small fish community structure in Ozark streams: structured assembly patterns or random abundance of species? Am. Midl. Nat. **107**: 42–54.

Menge, B.A., and Sutherland, J.P. 1976. Species diversity gradients: synthesis of the role of predation, competition and temporal heterogeneity. Am. Nat. **110**: 351–369.

Osborne, L.L., and Wiley, M.J. 1992. Influence of tributary spatial position on the structure of warmwater fish communities. Can. J. Fish. Aquat. Sci. **49**: 671–681.

Patterson, B.D., and Atmar, W. 1986. Nested subsets and the structure of insular mammalian faunas and archipelagos. Biol. J. Linn. Soc. **28**: 65–82.

Peterka, J.J. 1989. Fishes in northern prairie wetlands. *In* Northern prairie wetlands. *Edited by* A.D. van der Valk. Iowa State University Press, Ames, Iowa. pp. 302–315.

Poff, N.L., and Ward, J.V. 1989. Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. Can. J. Fish. Aquat. Sci. **46**: 1805–1818.

Power, M.E., Stout, R.J., Cushing, C.E., Harper, P.P., Hauer, F.R., Matthews, W.J., Moyle, P.B., Statzner, B., and Wais de Badgen, I.R. 1988. Biotic and abiotic controls in river and stream communities. J. North Am. Benthol. Soc. **7**: 456–479.

Prouty, W.F. 1952. Carolina bays and their origin. Geol. Soc. Am. Bull. **63**: 167–224.

Pulliam, H.R., and Danielson, B.J. 1991. Sources, sinks, and habitat selection: a landscape perspective on population dynamics. Am. Nat. **137**: S50–S66.

Rahel, F.J. 1984. Factors structuring fish assemblages along a bog lake successional gradient. Ecology, **65**: 1276–1289.

Richardson, C.J., and Gibbons, J.W. 1993. Pocosins, Carolina bays, and mountain bogs. *In* Biodiversity of the southeastern United States/lowland terrestrial communities. *Edited by* W.H. Martin, S.G. Boyce, and A.C. Echternacht. John Wiley & Sons, Inc., New York. pp. 257–310.

Schalles, J.F. 1989. Comparative chemical limnology of Carolina bay wetlands on the upper coastal plain of South Carolina. *In* Freshwater wetlands and wildlife. U.S. Department of Energy Conference Series No. CONF-8603101. *Edited by* R.R. Sharitz and J.W. Gibbons. U.S. Department of Energy, Washington, D.C. pp. 89–112.

Schalles, J.F., and Shure, D.J. 1989. Hydrology, community structure, and productivity of a dystrophic Carolina bay wetland. Ecol. Monogr. **59**: 365–385.

Schalles, J.F., Sharitz, R.R., Gibbons, J.W., Leversee, G.J., and

Knox, J.N. 1989. Carolina bays of the Savannah River Plant. National Environmental Research Park Program No. SRO-NERP-8. Savannah River Ecology Laboratory, Aiken, S.C.

Sharitz, R.R., and Gibbons, J.W. 1982. The ecology of southeastern shrub bogs (pocosins) and Carolina bays: a community profile. Project No. FWS/OBS-82/04. U.S. Fish and Wildlife Service, Division of Biological Services, Washington, D.C.

Sheldon, A. 1968. Species diversity and longitudinal succession in stream fishes. Ecology, **49**: 193–197.

Taylor, B.E., Mahoney, D.L., and Estes, R.A. 1989. Zooplankton production in a Carolina bay. *In* Freshwater wetlands and wildlife. U.S. Department of Energy Conference Series No. CONF-8603101. *Edited by* R.R. Sharitz and J.W. Gibbons. U.S. Department of Energy, Washington, D.C. pp. 425–435.

Tilly, L.J. 1973. Comparative productivity of four Carolina lakes. Am. Midl. Nat. **90**: 356–365.

Tonn, W.M., and Magnuson, J.J. 1982. Patterns in the species composition and richness of fish assemblages in northern Wisconsin lakes. Ecology, **63**: 1149–1166.

Wiens, J.A. 1984. On understanding a non-equilibrium world: myth and reality in community patterns and processes. *In* Ecological communities: conceptual issues and evidence. *Edited by* D.R. Strong, Jr., D. Simberloff, L.G. Abele, and A.B. Thistle. Princeton University Press, Princeton, N.J. pp. 439–457.

Wilkinson, L. 1987. SYSTAT: the system for statistics. SYSTAT, Inc., Evanston, Ill.

A review of size trends among North Pacific salmon (*Oncorhynchus* spp.)

Brian S. Bigler, David W. Welch, and John H. Helle

Abstract: The abundance of North Pacific salmon (*Oncorhynchus* spp.) has nearly doubled during the period 1975–1993. As salmon population numbers have increased, there have been corresponding decreases in average adult size at return (maturity). As nearly all of the growth of Pacific salmon occurs in the ocean, the ocean plays an important role in determining salmon abundance. We found that 45 of 47 North Pacific salmon populations, comprising five species from North America and Asia, are decreasing in average body size. Total salmon production correlated well with environmental trends between 1925 and 1989, but the inverse relationship between population abundance and average size during the period 1975–1993 indicates that there is a limitation to the salmon-sustaining resources of the ocean. The increased ocean survivorship and expansion of enhancement programs in the 1980s and early 1990s are probable factors in the ocean-wide reduced size of salmon. If these trends continue, the productivity of salmon populations may decrease as fecundity, egg size, and age at maturity change in response.

Résumé : L'abondance des saumons du Pacifique nord (*Oncorhynchus* spp.) a presque doublé au cours de la période de 1975 à 1993. À mesure que la population de saumons a croissé, on a noté une diminution correspondante de la taille moyenne des adultes lors de la remonte (à maturité). Comme presque toute la période de croissance des saumons se déroule dans l'océan, ce dernier joue un rôle important dans l'abondance de ces poissons. Nous avons constaté une diminution de la taille corporelle moyenne chez 45 des 47 populations de ces saumons, qui comprennent cinq espèces de l'Amérique du Nord et de l'Asie. La production totale de saumons était assez bien corrélée avec les tendances environnementales de 1925 à 1989, mais la relation inverse entre l'abondance de la population et la taille moyenne observée de 1975 à 1993 indique l'existence d'une limitation des ressources de l'océan supportant le saumon. Le taux de survie accru dans l'océan et l'élargissement des programmes de mise en valeur au cours des années 1980 et au début des années 1990 sont des facteurs probables de la réduction de la taille du saumon observée dans toutes les parties de l'océan. Si ces tendances persistent, la productivité des populations de saumons pourrait diminuer en réponse aux changements de la fécondité, de la taille des oeufs et de l'âge du poisson à maturité.
[Traduit par la Rédaction]

Introduction

Advances in population management, artificial enhancement, and favorable ocean conditions have more than doubled the population of salmon (*Oncorhynchus* spp.) in the North Pacific Ocean over the past 20 years (Rogers 1994) (Fig. 1). There is a growing body of evidence, however, that some North Pacific salmon populations have decreased in average size at maturity as ocean abundance has increased.

Studies of reduced growth among Pacific salmon have been relatively few and limited to individual spawning populations or species. Kaeriyama (1989, 1996), Kaeriyama and Urawa (1992), Ishida et al. (1993), and Hayashizaki and Hitoshi (1996) have documented decreases in the average size of Asian chum salmon (*Oncorhynchus keta)* in recent years. Similarly, Helle and Hoffman (1995) have documented age-specific declines in the average size of two North American chum salmon populations.

Because the ocean distributions of Pacific salmon populations overlap (Myers et al. 1990), significant trophic interaction among, and within, species could result in the expression of density-dependent growth if food is limiting. Some evidence exists for an inverse relationship between the growth of sockeye salmon (*Oncorhynchus nerka*) and the ocean abundance of that species (Rogers 1980; Peterman 1984). Similar findings have been reported for pink salmon (*Oncorhynchus gorbuscha*) (Foerster and Pritchard 1941; Davidson and Vaughan 1941; Ricker et al. 1978), coho salmon (*Oncorhynchus kisutch*) (Ricker and Wickett 1980; van den Berghe and Gross 1989), and chinook salmon (*Oncorhynchus tshawytscha*) (Ricker 1980, 1981). At least two hypotheses have been advanced that can explain declining body size: density-dependent growth, and selection of larger, older fish by selective fisheries.

While various authors have reported evidence that salmon size has decreased in the recent past, there is evidence of both increasing and decreasing average body size over longer periods. The age-specific size at maturity of chum salmon declined slightly from 1959 to 1978 in Prince William Sound, Alaska (Helle 1979). Godfrey (1959*a*) found that the average weight of British Columbia pink salmon increased from 1944 through 1958, and there was a similar increase in the average weight

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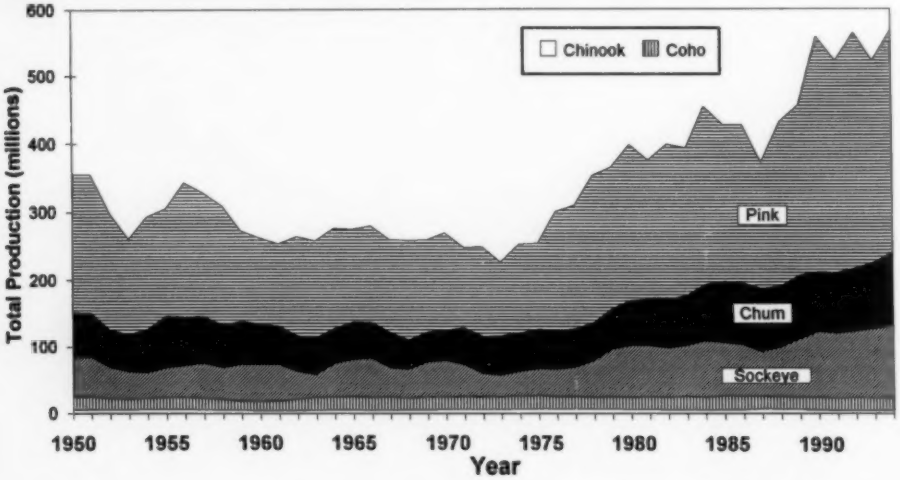
Fig. 1. Total North Pacific Ocean salmon production, 1951–1994. Data from D. Rogers, Fisheries Research Institute, University of Washington, Seattle, WA 98195.



of chum salmon from 1946 through 1958 (Godfrey 1959*b*). Conversely, Ricker et al. (1978) found that the average size of British Columbia pink salmon decreased subsequent to the period reported by Godfrey (1951–1974). Ricker (1981) reported coincident changes in age and decreasing size among all Pacific salmon species intercepted in the commercial fisheries of British Columbia since the early 1950s, but found differing results in a subsequent study (Ricker 1994).

As growth rates decrease, many life history traits that affect population productivity also decrease (Forbes and Peterman 1994). Reduction in fecundity and egg size, increases in mean age at reproduction, or the ability to migrate upstream and spawn with optimal success all influence population productivity and therefore harvest rates. Helle (1989), for example, found that the mean size (length) of chum salmon spawners is positively related to the survival of their progeny. If this relationship applies more generally, declines in size could reduce survival. We present a survey of reported associations between growth rate and other density-related population responses in Pacific salmon. We also examine temporal trends in size and age and review data on fecundity and egg size.

Materials and methods

A variety of state, provincial, and federal governmental agencies in North America provided average weight and other data for five Pacific salmon species to test for trends in average size (Fig. 2). Additional summary data were available from published reports (Ishida et al. 1993; Welch and Noakes 1993). Pacific salmon abundance nearly doubled in the period 1975–1993 compared with that in the period 1950–1975 (Fig. 1). Analyses are confined to data characterizing the years 1975–1993.

Several authors reporting on temporal changes in body size favor linear regression analysis (e.g., Ricker 1981; Kaeriyama 1989; Ishida et al. 1993). Fundamental to the generation of unbiased regression statistics is the assumption that error terms are random. Because salmon return to spawning rivers after 1 to several years at sea, those harvested in nearshore fisheries comprise portions of brood cohorts resulting from several spawning seasons. Though not true in every case, it is not unusual for the year to year average size of salmon to follow cyclical, nonrandom patterns over time. Data that follow such patterns are often autocorrelated and do not always fit a linear regression model without inefficient estimation of slope and underestimation of slope standard error (J. Pella, National Marine Fisheries Service, 11 305 Glacier Highway, Juneau, AL 99801, personal communication). As a result, the risk of rejecting the null hypothesis (that slope is zero) is higher. The simple linear regression model with the random error terms following an autoregressive process is $Y_t = \beta_0 + \beta_1 X_t + \varepsilon_t$, where $\varepsilon_t = \rho\varepsilon_{t-1} + u_t$. Each error term ε_t in this model consists of a fraction of the previous error term $\rho\varepsilon_{t-1}$ plus a disturbance term u_t.

Autocorrelation was tested following the procedure of Neter et al. (1985) to calculate unbiased regression statistics. The slope of generated regressions, expressed as *b* throughout this document, is the calculated average annual change in the variable tested.

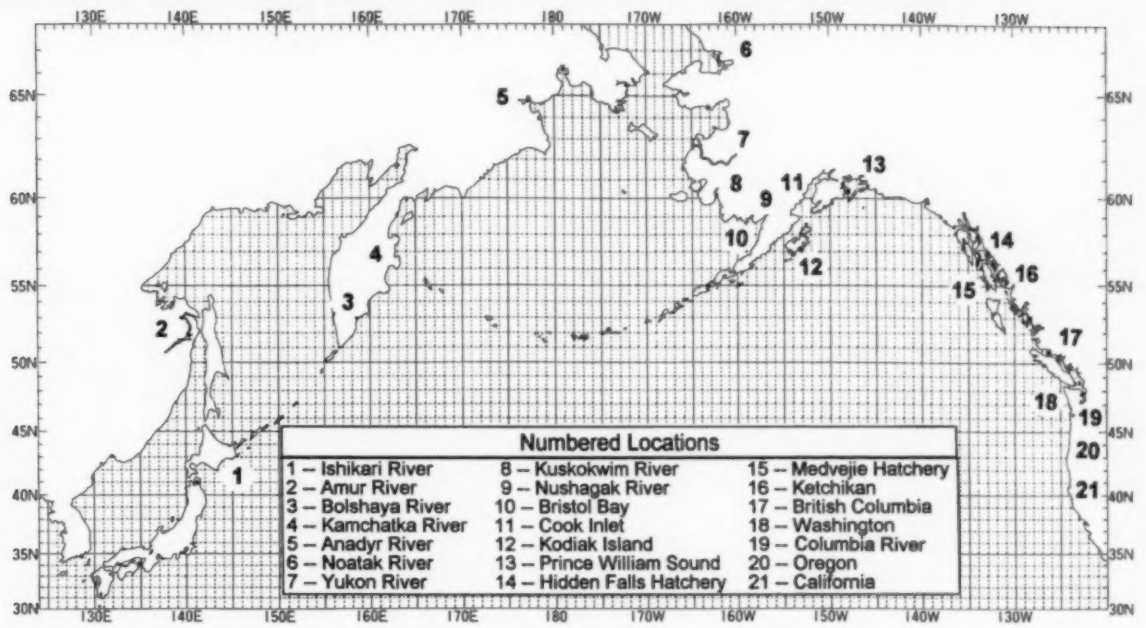
Data sources

Size-at-return data (weight or length) are based on summaries of commercial harvest information collected by state and federal government agencies, and data summaries from the Pacific Biological Station, Nanaimo, British Columbia (Welch and Noakes 1993), and the University of Washington (Rogers 1994). Chum salmon fecundity and egg size data were supplied by state and private hatcheries in Oregon and Alaska. Other unique data sources are identified when referenced. Where possible, data from less selective fishing gear (seine, troll) were used.

The Pacific Fishery Management Council Review of Fisheries summarizes data on dressed weight of troll-caught chinook and coho in the states of California, Oregon, and Washington (Rod Kaiser, Oregon Department of Fish and Wildlife, 2040 Southeast Marine Science Drive, Newport, OR 97365, personal communication). California, Oregon, and Washington troll fishery data for the month of August were selected for analysis because chinook average weights were highest during August, indicating that mature salmon comprised the highest proportion of the catch, and because data are available for all years.

The Oregon Department of Fish and Wildlife (ODFW) provided data on average size at return, fecundity, and egg size for Columbia River salmon returning to hatcheries (John Leppink, Hatchery Data

Fig. 2. Locations of salmon populations tested for changes in average weight, length, age, or ancillary information.



Coordinator, ODFW, P.O. Box 59, Portland, OR 97207, personal communication). The average sizes of salmon in Columbia River commercial harvests were taken from published data (Oregon Department of Fish and Wildlife 1993).

Welch and Noakes (1993) reported average size data for all salmon species in British Columbia. Ricker (1994) reported a comprehensive analysis of these data.

The Alaska Department of Fish and Game provided average weight data for each species harvested in the following nearshore commercial fisheries: Kotzebue Sound, Yukon River, Kuskokwim River, Bristol Bay, Kodiak Island, Cook Inlet, and Ketchikan (Herman Savikko, Alaska Department of Fish and Game, P.O. Box 25526, Juneau, AL 99802-5526, personal communication).

Several hatcheries in Alaska provided average size, fecundity, and egg size data (Steve McGee, Alaska Department of Fish and Game, P.O. Box 25526, Juneau, AL 99802-5526, personal communication; Bruce Bachen, Northern Southeast Regional Aquaculture Association, 1308 Sawmill Creek Road, Sitka, AL 99835, personal communication; Peter Rob, Sikusuilaq Springs Hatchery, P.O. Box 1030, Kotzebue, AL 99752, personal communication). Sikusuilaq Springs Hatchery on the Noatak River in northwest Alaska has produced fall chum salmon since 1982. Hidden Falls and Medvejie hatcheries in southeast Alaska provided fecundity data collected since 1986 and 1988, respectively. The Port San Juan Hatchery in Prince William Sound, Alaska, provided 14 years of average fecundity information.

Decreases in average size for chum salmon populations can be confounded by variability in age at return. An increasing number of older, larger fish (Helle and Hoffman 1995) will tend to mask the overall decrease in average body size. Age-specific size data covering the period under review are not generally available. Pink salmon return at 2 years of age and coho salmon generally return at age 3; however, there is probably little likelihood that variation in age at return will be confounded with variation in growth rate because these species always spend only one summer (two winters) at sea. Chinook, sockeye, and chum salmon return to natal streams following one to several winters at sea. There are sufficient data to examine changes in age-specific growth rates for chinook from the Yukon, Kuskokwim, and Kenai rivers, Yukon River fall and summer chum, and Bristol Bay and Kenai River sockeye.

A decrease in average body size results in a smaller volume and decreasing fecundity (Bagenal 1969; Beacham and Murray 1987; Helle 1989; Fleming and Gross 1990). Although a direct relation with body size (weight) is assumed, fecundity and egg size are not regularly measured for wild salmon populations in western Alaska. Data from Columbia River coho, Sikusuilaq Springs (Noatak River; Kotzebue, Alaska) chum and Hidden Falls and Medvejie (southeast Alaska) chum, and Prince William Sound pink salmon hatchery populations are available (Fig. 2).

Results

Among five species of Pacific salmon, 45 of 47 populations tested decreased in mean size between 1975 and 1993 (Table 1, Figs. 3–7). Only California and British Columbia chinook salmon failed to show an inverse association between size and time.

Chinook salmon

Chinook salmon migrate to sea after spending up to two winters in fresh water; they then return to spawn zero to five winters later. Among nine populations of chinook salmon tested, the calculated average weights of chinook in California and British Columbia increased 25 and 45%, respectively, between 1975 and 1993 (Table 1, Fig. 3). Body size decreased in all other populations tested, from 10 to 47%. The decline in body weight was least for Oregon chinook (0.047 kg/year) and greatest for Kuskokwim River chinook (0.162 kg/year) (Table 1).

Age-specific length data collected with the Yukon River commercial fisheries and Kenai River sport fishery corroborate

Table 1. Trends in mean weight (kg) of North Pacific salmon populations.

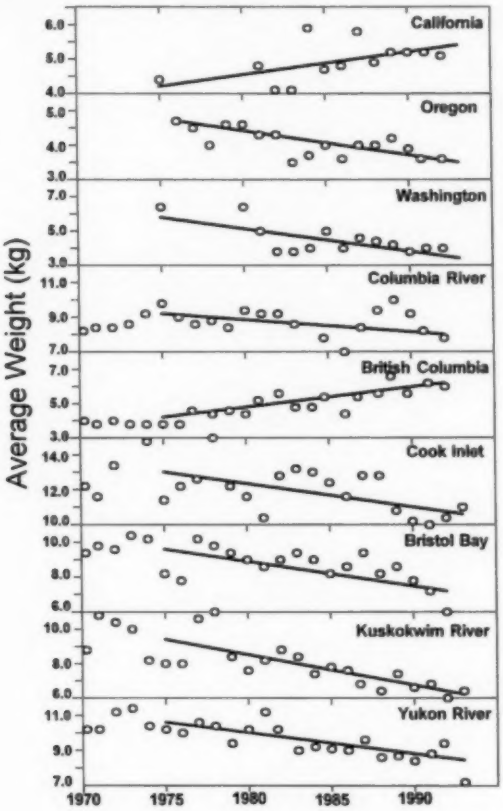
Region	Area or fishery	*n*	*r*	*b*	Calculated weight 1975	Calculated weight 1993	% change
			Chinook				
California	Troll fishery	12	0.439	0.058	4.21	5.25	24.70
Oregon	Troll fishery	17	−0.627**	−0.047	4.61	3.76	−18.44
Washington	Troll fishery	14	−0.747**	−0.161	6.21	3.31	−46.70
	Columbia River	18	−0.568*	−0.054	9.16	8.19	−10.09
British Columbia	Troll fishery	18	0.761**	0.105	4.15	6.04	45.54
Alaska	Cook Inlet	19	−0.566*	−0.106	12.82	10.91	−14.90
	Bristol Bay	19	−0.637**	−0.136	9.59	7.14	−25.55
	Kuskokwim River	19	−0.761**	−0.162	9.27	6.35	−31.50
	Yukon River	19	−0.670**	−0.102	10.43	8.60	−17.55
			Coho				
California	Troll fishery	12	−0.316	−0.024	3.17	2.74	−13.56
Oregon	Troll fishery	17	−0.588*	−0.043	2.84	2.07	−27.11
Washington	Troll fishery	14	−0.693**	−0.044	2.56	1.77	−30.86
	Columbia River	18	−0.737**	−0.059	3.90	2.83	−27.44
British Columbia	Troll fishery	18	−0.600**	−0.035	3.15	2.52	−20.00
Alaska	Kodiak	19	−0.500*	−0.025	4.01	3.57	−10.97
	Bristol Bay	19	−0.378	−0.017	3.46	3.16	−8.67
	Kuskokwim River	19	−0.560*	−0.018	3.40	3.07	−9.71
	Yukon River	17	−0.374	−0.012	3.21	2.99	−6.85
			Chum				
Washington	Columbia River	18	−0.564*	−0.075	6.12	4.84	−20.92
British Columbia	Seine or gill net	18	−0.521*	−0.046	5.45	4.63	−15.05
Alaska	Ketchikan	19	−0.570*	−0.035	4.50	3.87	−14.00
	Cook Inlet	19	−0.627**	−0.029	3.64	3.10	−14.84
	Kodiak	19	−0.637**	−0.036	3.92	3.28	−16.33
	Bristol Bay	19	−0.280	−0.005	3.00	2.91	−3.00
	Kuskokwim River	19	−0.460*	−0.013	3.26	3.02	−7.36
	Yukon River (summer)	16	−0.144	−0.003	3.22	3.17	−1.55
	Yukon River (fall)	16	−0.765**	−0.030	3.59	3.02	−15.88
	Kotzebue Sound (fall)	19	−0.545*	−0.018	4.13	3.80	−7.99
Russia	Anadyr River	11	−0.720*	−0.083	4.69	3.20	−31.77
	Kamchatka River	16	−0.650**	−0.029	3.88	3.35	−13.66
	Bolshaya River	15	−0.502	−0.039	4.02	3.33	−17.16
	Amur River (summer)	16	−0.546*	−0.016	2.66	2.37	−10.90
	Amur River (fall)	18	−0.627**	−0.048	4.89	4.03	−17.59
Japan	Ishikari River	16	−0.646**	−0.048	3.72	2.85	−23.39
			Pink				
British Columbia	Seine or gill net	18	−0.505*	−0.030	1.97	1.43	−27.41
Alaska	Ketchikan	19	−0.710**	−0.030	1.89	1.36	−28.04
	Prince William Sound	15	−0.529*	−0.015	1.64	1.37	−16.46
	Cook Inlet	19	−0.760**	−0.020	1.73	1.37	−20.81
	Kodiak	19	−0.722**	−0.021	1.82	1.45	−20.33
	Bristol Bay	19	−0.676**	−0.020	1.91	1.56	−18.32
			Sockeye				
British Columbia	Seine or gill net	18	−0.039	−0.002	2.58	2.55	−1.16
Alaska	Ketchikan	19	−0.315	−0.008	2.77	2.64	−4.69
	Cook Inlet	19	−0.474*	−0.027	3.05	2.70	−11.48
	Kodiak	19	−0.665**	−0.027	2.85	2.37	−16.84
	Bristol Bay	19	−0.136	−0.003	2.71	2.66	−1.85

Note: *n*, number of years; *r*, correlation coefficient; *b*, regression slope (average annual change in weight). Predicted values for 1975 and 1993 are based on linear regression, and were used to calculate the percent change. Data for chum salmon from Russia and Japan are taken from Ishida et al. (1993), and predicted values are for the years 1970 and 1988.

* $P < 0.05$

** $P < 0.01$.

Fig. 3. Mean weight of chinook salmon sampled from nine North American commercial fisheries.



these findings (Table 2, Fig. 8). All age groups tested decreased by approximately 3–5% of body length.

The mean age at return of Kuskokwim River chinook salmon decreased over the period 1975–1993, while the age of Yukon River chinook remained essentially unchanged and Kenai River chinook increased in mean age over the period examined (Table 2, Fig. 8).

Coho salmon

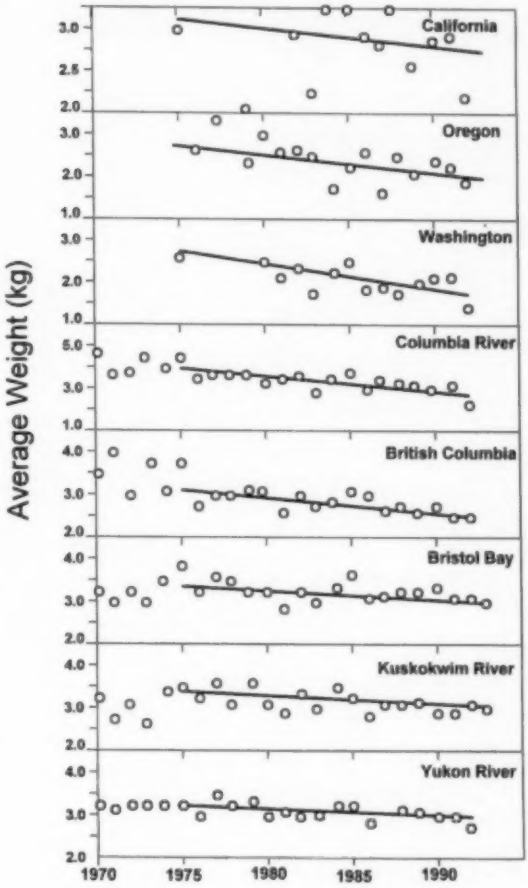
The majority of coho salmon are captured during their third or fourth year of life after spending two winters, or one summer, at sea. All populations tested showed decreased average body weight ranging from –0.012 to –0.059 kg/year (Table 1, Fig. 4).

Chum salmon

Chum salmon characteristically migrate to sea soon after fry emergence, and return from two to five winters later. Chum salmon among the 10 populations examined declined in average weight from 0.003 kg/year for Yukon River summer chum to 0.075 kg/year for Columbia River chum (Table 1, Fig. 5).

Age-specific data for Yukon River fall and summer chum show that as average size at age decreased, the mean age at return increased. The average lengths of each age group of

Fig. 4. Mean weight of coho salmon sampled from eight North American commercial fisheries.



summer and fall chum decreased similarly, at rates of 2–4% over 1975–1993 (Table 3, Fig. 9). Summer chum age increased from 3.0 to 3.8 years, while fall chum increased from 2.9 to 3.4 years.

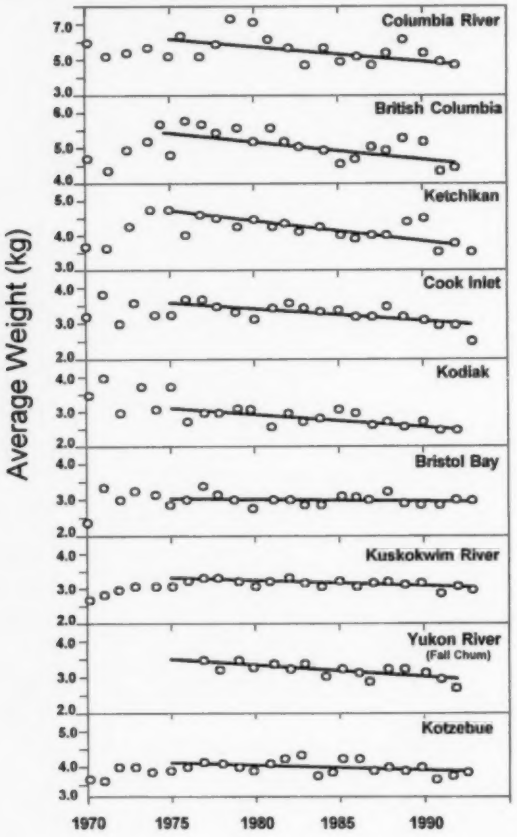
Pink salmon

Pink salmon follow a 2-year life cycle, spending one winter at sea. The average weights of North American pink salmon populations have decreased at a relatively uniform rate of approximately 0.020 kg/year, or about 20% from 1975 to 1993 (Table 1, Fig. 6). The decline is most notable in British Columbia where the declining trend has continued since the early 1950s (Ricker et al. 1978); currently British Columbia pink salmon are approximately 1 kg (40%) smaller than in the 1950s (Ricker 1994).

Sockeye salmon

The sockeye salmon life history is very plastic, with fish spending from zero to three winters in fresh water and one to four winters at sea. Though regression slopes are uniformly negative, only two of five sockeye salmon populations tested

Fig. 5. Mean weight of chum salmon sampled from nine North American commercial fisheries.



showed statistically significant ($P < 0.05$) declines (Table 1, Fig. 7). Sockeye salmon harvested from Kodiak Island and Cook Inlet decreased by 0.027 kg/year (16.8 and 11.5%, respectively) from 1975 to 1993.

Age-specific length measurements for sockeye salmon caught in the Cook Inlet and Bristol Bay gill-net fisheries show similar tendencies in declining length and weight, respectively (Table 4, Fig. 10). The average ocean age of these sockeye salmon gradually increased as growth rates declined and a smaller proportion of young fish returned with time (Table 4).

Fecundity and egg size

Fecundity and egg size data collected from hatchery populations are well suited for basic comparison with wild salmon stocks. Fecundity declined among all hatchery populations tested (Fig. 11). The egg sizes of coho salmon and fall and summer chum showed a significant decrease with decreasing fecundity (Fig. 12).

Discussion

Maximization of natural production and advances in artificial enhancement techniques have assisted in the near doubling of salmon harvests over the past two decades. As North Pacific

Fig. 6. Mean weight of pink salmon sampled from six North American commercial fisheries.

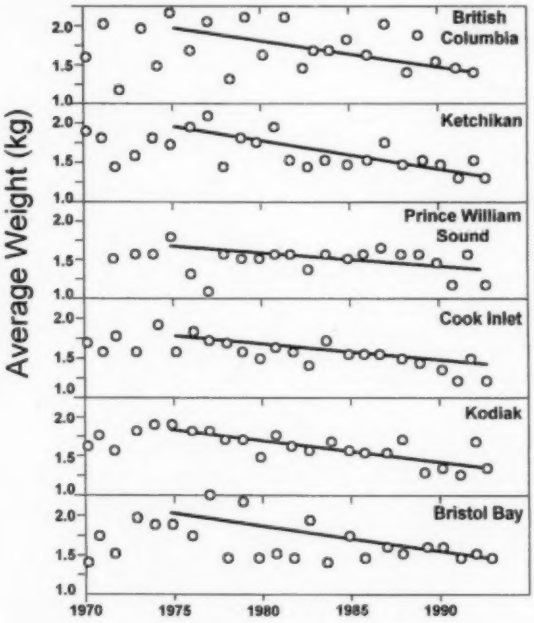
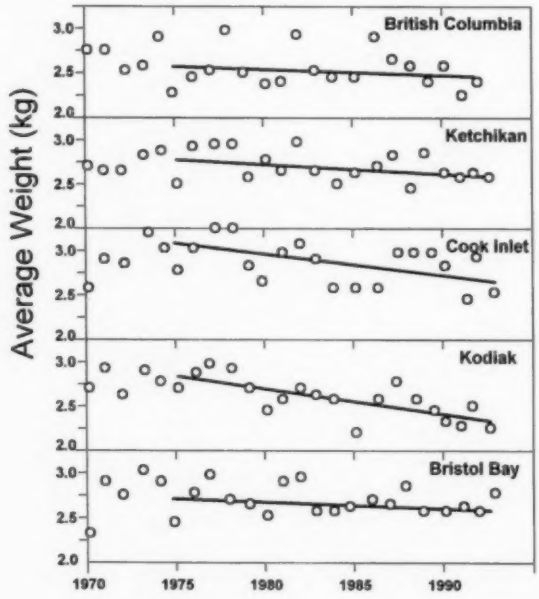


Fig. 7. Mean weight of sockeye salmon sampled from five North American commercial fisheries.



salmon population numbers have expanded, there has been a coincident decrease in average adult size and an increase in average age at maturity.

The changes in both average size and age for western

Table 2. Trends in age-specific mean length (mm) and average age (years) of selected North Pacific chinook salmon populations.

Population	Age group	*n*	*r*	*b*	Calculated values 1975	1979	1993	% change
				Length				
Yukon River	3-ocean	14	−0.658*	−2.16		789.9	759.7	−3.82
	4-ocean	14	−0.485	−1.74		885.0	860.7	−2.75
	5-ocean	13	−0.508	−4.11		965.5	912.1	−5.53
Kenai River	3-ocean	17	−0.444	−4.25	905.0		828.5	−8.50
	4-ocean	17	−0.742**	−6.52	1102.5		985.0	−10.70
	5-ocean	17	−0.545*	−5.88	1169.4		1063.6	−9.00
				Age				
Yukon River	Ocean age	19	0.078	0.002	3.69		3.77	2.17
Kuskokwim River	Ocean age	19	−0.821**	−0.049	3.64		2.76	−24.18
Kenai River	Ocean age	16	−0.731**	−0.039	3.30		4.00	20.90

Note: *n*, number of years considered; *r*, correlation coefficient; *b*, regression slope (average annual change in weight). The calculated values (lengths for the length section of the table, and ages for the age section of the table) were computed from available data.

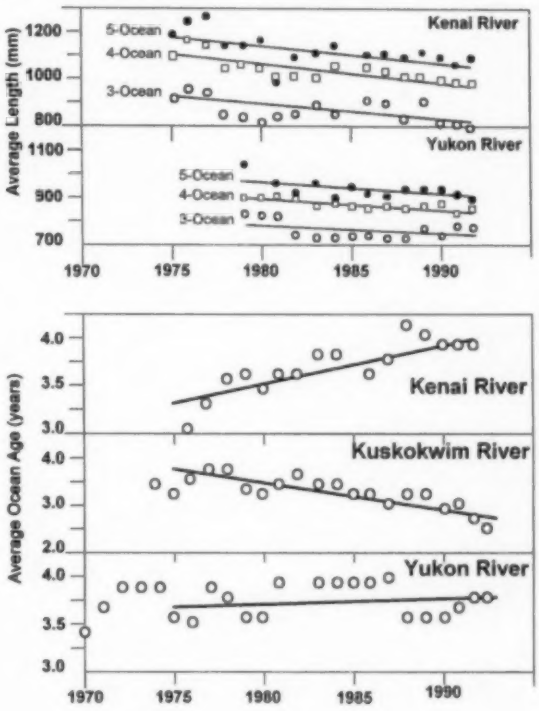
*$P < 0.05$.

**$P < 0.01$.

Alaska chum salmon mirror those for Japanese and Russian stocks reported by Ishida et al. (1993), and for two other North American populations reported by Helle and Hoffman (1995). Ishida et al. (1993) observed a depressed growth rate during the third year of life among both Japanese and Russian chum salmon and concluded that density-dependent factors explained 35% of the resultant decrease in average size. The remaining 65% was attributable to other factors, including artificial enhancement and interactions with other salmon species. Helle (1979) showed that reduced growth of Prince William Sound chum salmon during the second year at sea delayed the onset of maturity. Chum experience reduced food availability during periods of large population numbers, and reduced growth delays the onset of sexual maturation. Although pink salmon have maintained a fixed 2-year age at return, the declines in mean size have been even larger than for chum salmon. Similar changes are evident for other salmon species, although the evidence for widespread declines in growth rate is not as consistent as for pink and chum salmon. Sockeye salmon sampled from two locations in Alaska also exhibited an increase in average ocean age as average size decreased. The results for the average age of chinook salmon examined here are ambiguous. The mean age of Kuskokwim River chinook has decreased, the age of Yukon River chinook has been relatively unchanged, and Kenai River chinook have increased in age over the period examined. Reduction in average age coincident with average weight of British Columbia chinook was also reported by Ricker (1981) and is probably a result of differential exposure to mortality during the ocean residence. Comprehensive estimates of chinook salmon bycatch by vessels operating in both Alaskan and Russian waters of the Bering Sea are not available. Within the U.S. Exclusive Economic Zone in the Bering Sea the annual interception of chinook salmon is approximately 50 000 fish/year (G. Trumble, National Marine Fisheries Service, Juneau, AL 99801, personal communication). This level of removal is probably too small to influence estimates of average size or age.

Each of the above cases of decreasing size with time may be the result of increasing salmon abundance causing a reduction in the available food supply through density-dependent interaction and retarding size and age. Brodeur (1990) found

Fig. 8. Change in average length and age among chinook salmon from the Kuskokwim, Yukon, and Kenai rivers.



significant dietary overlap between chinook, coho, pink, and sockeye salmon, and that these species appear to feed opportunistically on a broad range of prey organisms. Chum salmon appear to be the most specialized and select food items not commonly shared with other species. Ricker (1981), on the other hand, attributed the decline in growth of British Columbia salmon to size-selective fisheries acting to select against rapid growth and discounted the possibility of environmental or density-dependent factors playing a major role. The use of

Table 3. Trends in age-specific mean length (mm) and average age (years) of selected North Pacific chum salmon populations.

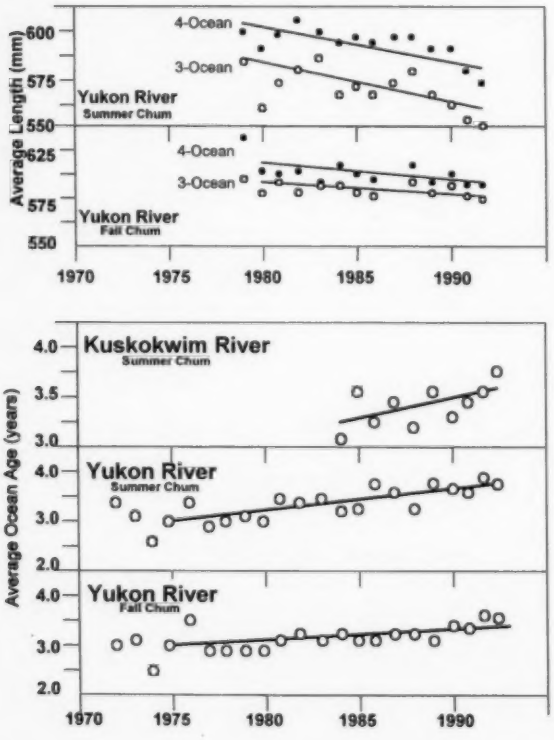
Population	Age group	*n*	*r*	*b*	Calculated values 1975	1979	1993	% change
			Length					
Yukon River (summer)	0.3	14	−0.587*	−1.19		580.4	563.7	−2.88
	0.4	14	−0.782**	−1.82		605.1	579.6	−4.21
Yukon River (fall)	0.3	13	−0.534*	−0.71		598.1	588.1	−1.67
	0.4	13	−0.704**	−1.18		619.1	602.6	−2.67
			Age					
Yukon River (summer)	Ocean age	19	0.805**	0.041	3.03		3.77	18.36
Yukon River (fall)	Ocean age	19	0.629**	0.028	2.92		3.42	12.76
Kuskokwim River (summer)	Ocean age	10	0.453	0.033	nc		nc	nc
Anadyr River	Ocean age	11	0.265	0.014	3.16		3.41	6.01
Kamchatka River	Ocean age	16	0.665**	0.025	3.15		3.60	10.84
Bolshaya River	Ocean age	15	0.611*	0.039	2.96		3.66	17.68
Amur River (summer)	Ocean age	16	0.817**	0.027	2.80		3.28	12.63
Amur River (fall)	Ocean age	18	0.823**	0.024	2.80		3.23	11.32
Ishikari Rivera	Ocean age	16	0.574*	0.028	2.31		2.82	15.41

Note: *n*, number of years considered; *r*, correlation coefficient; *b*, regression slope (average annual change in weight); nc, not calculated. The calculated values (lengths for the length section of the table, and ages for the age section of the table) were computed from available data. Data for the Anadyr, Kamchatka, Boshaya, Amur, and Ishikari rivers are from Ishida et al. (1993).

*$P < 0.05$.

**$P < 0.01$.

Fig. 9. Change in average length and age among chum salmon from the Kuskokwim and Yukon rivers.



selective gear types in nearshore harvests may contribute to the reduced average size of salmon through a genetic response, but it is highly unlikely that commercial fishing pressures throughout the North Pacific species range are acting simultaneously to account for the decreasing size among all species. Another possibility is that the increases in salmon abundance over the last two decades could be the result of reduced mortality on slower growing members of a cohort; in this case, changes in survival act to increase the proportion of small, slow-growing fish contributing to the fishery, thereby lowering the average size at age, even if growth rates do not change.

Environmental influences

Changes in population parameters that are coincident on an oceanwide scale are probably caused by common events during the ocean phase. Beamish and Bouillon (1993) found that long-term changes in the intensity of the Aleutian low pressure system, a weather system extending throughout the southern Bering Sea and North Pacific Ocean, correlate well with fluctuations in salmon abundance and the production of copepods. The inverse relationship between population abundance and average body size during the same period, however, suggests that there may be a limitation to the salmon-sustaining resources of the ocean.

Implications of reduced body size

Reduction in body size may result in reduced reproductive success (Helle 1989; review by Forbes and Peterman 1994). Life history theory predicts that large body size is a premium among salmon populations that migrate over long distances to spawn and enter the ocean as smolt. Beacham et al. (1988) and Beacham and Murray (1987) conclude that salmon body shape is heavily influenced by local selective forces in large and small rivers. Chum salmon spawning in large rivers in British Columbia adapt larger heads, thicker caudal peduncles, and larger fins than those spawning in smaller rivers. Healey and Heard (1984) found that egg numbers and adult body length are positively correlated in chinook. They concluded that there is substantial variation in fecundity between populations as an

Table 4. Trends in age-specific mean length (mm), weight (kg), and average age (years) of selected North Pacific sockeye salmon populations.

Population	Age group	n	r	b	Calculated values 1975	Calculated values 1993	% change
			Weight				
Bristol Bay	2-ocean	20	−0.671**	−0.013	2.41	2.18	−23.00
	3-ocean	20	−0.663**	−0.018	3.25	2.94	−31.60
			Length				
Cook Inlet	2-ocean	17	−0.675**	−1.85	522.0	488.7	−6.38
	3-ocean	17	−0.780**	−2.47	596.4	552.0	−7.44
			Age				
Bristol Bay	Ocean	20	0.296	0.009	2.34	2.48	14.21
Cook Inlet	Ocean	19	0.420	0.010	2.61	2.79	6.90

Note: n, number of years considered; r, correlation coefficient; b, regression slope (average annual change in weight). The calculated values (weight, length, and age for the weight, length, and age sections of the table, respectively) were computed from available data.

$^{**}P < 0.01$.

Fig. 10. Change in average weight, length, and age among Bristol Bay and Cook Inlet sockeye salmon.

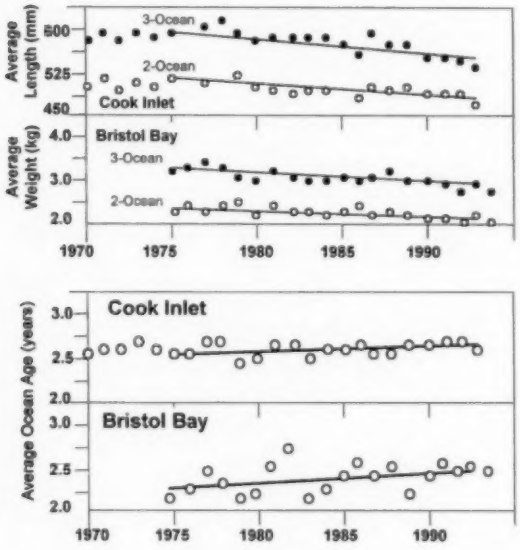
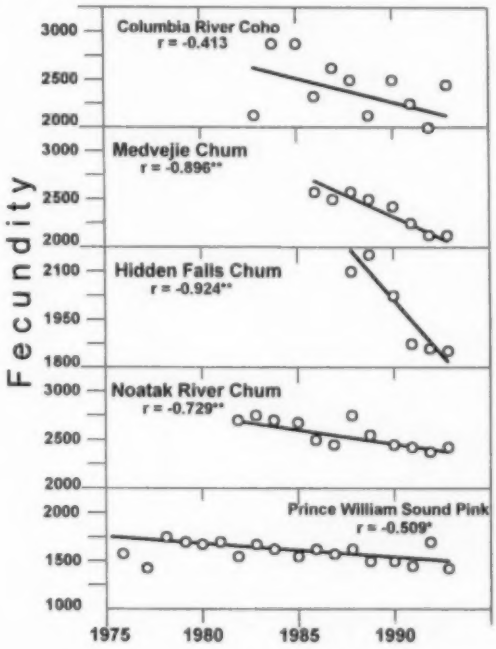


Fig. 11. Mean fecundity of hatchery coho, chum, and pink salmon, 1982–1993.

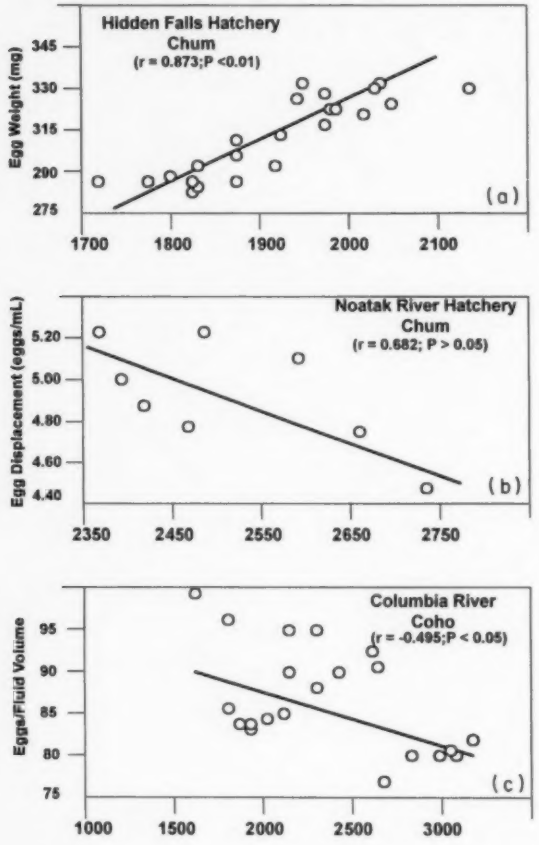


adaptation to specific spawning and habitat conditions. Healey (1982) demonstrated the importance of size for the survival of small salmon, a principle that is presumed true for larger salmon as well.

The reproductive value of size is attributable to physical strength and longevity on the spawning ground, as well as to advantages in the numbers and vitality of sexual products (egg size, sperm motility). Foerester and Pritchard (1941) showed that sockeye and pink salmon follow principles governing the relationship between fecundity and body size similar to those of other species. Specifically, decreased body size and the attendant reductions in fecundity and egg size equate to greatly reduced abundance and survivorship of the progeny. Beacham and Murray (1987) showed that small eggs produce smaller alevin and fry that in turn inherit a diminished probability of survival. The effects of reduced average size among salmon may first be evident in populations in which large body size is an important adaptation.

Average size data extending for more than several decades are rare. Ricker (1994) reported several cycles of increasing and decreasing size tendencies among British Columbia sockeye back to 1912. Welch and Morris (1994) found evidence of long-term density-dependent changes in average size among British Columbia pink salmon for the period 1927–1993. Such fluctuations probably occurred because of natural population pressures independent of influences from enhancement programs. The remarkable expansion of Pacific salmon enhancement programs since 1975, however, precludes simple comparison of the changes in average size documented here

Fig. 12. Relationship between egg size and fecundity in summer chum (*a*), fall chum (*b*), and coho (*c*) salmon, based on data from hatcheries in Alaska and Washington state.



with anything that has occurred previously. Artificially propagated salmon enjoy several survival advantages over wild populations, such as feeding to attain optimal fry size and abundance independent of environmental influence. Consequently, deleterious effects of reductions in average adult size will very likely appear first, and be most pronounced, among wild populations. As programs for the artificial propagation of Pacific salmon expand and the technology improves, the allocation of increasingly limited ocean resources may need to be recognized to assure optimal common management of salmon.

References

Bagenal, T.B. 1969. Relationship between egg size and fry survival in brown trout *Salmo truta* L. J. Fish Biol. **1**: 349–353.

Beacham, T.D., and Murray, C.B. 1987. Adaptive variation in body size, age, morphology, egg size, and developmental biology of chum salmon (*Oncorhynchus keta*) in British Columbia. Can. J. Fish. Aquat. Sci. **44**: 244–261.

Beacham, T.D., Withler, R.E., Murray, C.B., and Barner, L.W. 1988. Variation in body size, morphology, egg size, and biochemical genetics of pink salmon in British Columbia. Trans. Am. Fish. Soc. **117**: 109–125.

Beamish, R.J., and Bouillon, D.R. 1993. Pacific salmon production trends in relation to climate. Can. J. Fish. Aquat. Sci. **50**: 1004–1006.

Brodeur, R.D. 1990. A synthesis of the food habits and feeding ecology of salmonids in marine waters of the North Pacific. INPFC Doc. No. FRI-UW-9016. Available from K. Myers, Fisheries Research Institute, University of Washington, Seattle, WA 98195.

Davidson, F.A., and Vaughan, E. 1941. Relation of population size to marine growth and time of spawning migration in pink salmon (*Oncorhynchus gorbuscha*) of southeastern Alaska. J. Mar. Res. **4**: 231–246.

Fleming, I.A., and Gross, M.R. 1990. Latitudinal clines: a trade-off between egg number and size in Pacific salmon. Ecology, **71**: 1–11.

Foerester, R.E., and Pritchard, A.L. 1941. Observations on the weight relation of egg content to total length and weight in the sockeye salmon (*Oncorhynchus nerka*) and the pink salmon (*O. gorbuscha*). Trans. R. Soc. Can. **35**: 51–60.

Forbes, L.S., and Peterman, R.M. 1994. Simple size-structured models of recruitment and harvest in Pacific salmon (*Oncorhynchus* spp.). Can. J. Fish. Aquat. Sci. **51**: 603–616.

Godfrey, H. 1959*a*. Variations in annual average weights of British Columbia pink salmon 1944–1958. J. Fish. Res. Board Can. **16**: 329–337.

Godfrey, H. 1959*b*. Variation in the annual average weight of chum salmon caught in British Columbia waters, 1946–1958. J. Fish. Res. Board Can. **16**: 553–554.

Hayashizaki, K.I., and Hitoshi, I. 1996. Size decrease of chum salmon, *Oncorhynchus keta* in Tohoku districts, Japan. *In* Proceedings of the International Symposium on Biological Interactions of Enhanced and Wild Salmonids. *Edited by* B.E. Riddell and C.C. Woods. Can. Spec. Publ. Fish. Aquat. Sci. In press.

Healey, M.C. 1982. Timing and relative intensity of size selective mortality of juvenile chum salmon (*Oncorhynchus keta*) during early sea life. Can. J. Fish. Aquat. Sci. **39**: 952–957.

Healey, M.C., and Heard, W.R. 1984. Inter- and intra-population variation in the fecundity of chinook salmon (*Oncorhynchus tshawytscha*) and its relevance to life history theory. Can. J. Fish. Aquat. Sci. **41**: 476–483.

Helle, J.H. 1979. Influence of marine environment on age and size at maturity, growth, and abundance of chum salmon, *Oncorhynchus keta* (Walbaum), from Olsen Creek, Prince William Sound, Alaska. Ph.D. thesis, Oregon State University, Corvallis, Oreg. University Microfilms Int. No. 7927127.

Helle, J.J. 1989. Relation between size-at-maturity and survival of progeny in chum salmon, *Oncorhynchus keta* (Walbaum). J. Fish Biol. **35**(Suppl. A): 99–107.

Helle, J.H., and Hoffman, M.S. 1995. Size decline and older age at maturity of two chum salmon (*Oncorhynchus keta*) stocks in western North America, 1972–1992. *In* Climate change and northern fish populations. *Edited by* R.J. Beamish. Can. Spec. Publ. Fish. Aquat. Sci. No. 121. pp. 243–250.

Ishida, Y., Ito, S., Kaeriyama, M., McKinnell, S., and Nagasawa, K. 1993. Recent changes in age and size of chum salmon (*Oncorhynchus keta*) in the North Pacific Ocean and possible causes. Can. J. Fish. Aquat. Sci. **50**: 290–295.

Kaeriyama, M. 1989. Aspects of salmon ranching in Japan. Physiol. Ecol. Jpn. Spec. Vol. 1. pp. 625–638.

Kaeriyama, M. 1996. Production trends of salmon enhancement in Japan. *In* Proceedings of International Symposium on Biological Interactions of Enhanced and Wild Salmonids. *Edited by* B.E. Riddell and C.C. Woods. Can. Spec. Publ. Fish. Aquat. Sci. In press.

Kaeriyama, M., and Urawa, S. 1992. Future research by the Hokkaido Salmon Hatchery for the proper maintenance of Japanese salmonid stocks. *In* Proceedings of the International Workshop on Future Salmon Research in the North Pacific Ocean, Shimizu,

Japan,November 11, 1992. *Edited by* Y. Ishida, K. Nagasawa, D.W. Welch, K.W. Myers, and A.P. Shershnev. National Research Institute of Far Seas Fisheries, Shimizu, Japan. pp. 57–62.

Myers, K.W., Walker, R.V., Fowler, S., and Dahlberg, M.L. 1990. Known ocean ranges of stocks of Pacific salmon and steelhead as shown by tagging experiment, 1956–1989. INPFC Doc. No. FRI-UW-9009. Available from K. Myers, Fisheries Research Institute, University of Washington, Seattle, WA 98195.

Neter, J., Wasserman, W., and Kutner, M.H. 1985. Applied linear statistical models. R.D. Irwin, Inc., Homewood, Ill.

Oregon Department of Fish and Wildlife. 1993. Status report: Columbia River fish runs and fisheries, 1938–92. Oregon Department of Fish and Wildlife, 2040 Southeast Marine Science Drive, Newport, OR 97365.

Peterman, R.M. 1984. Density-dependent growth in early ocean life of sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. **41**: 1825–1829.

Ricker, W.E. 1980. Causes of the decrease in age and size of chinook salmon (*Oncorhynchus tshawytscha*). Can. Tech. Rep. Fish. Aquat. Sci. No. 944.

Ricker, W.E. 1981. Changes in the average size and average age of Pacific salmon. Can. J. Fish. Aquat. Sci. **38**: 1636–1656.

Ricker, D.E. 1994. Trends in the average size of Pacific salmon in Canadian catches. *In* Climate change and northern fish populations. *Edited by* R.J. Beamish. Can. Spec. Publ. Fish. Aquat. Sci. No. 121. pp. 593–602.

Ricker, W.E., and Wickett, W.P. 1980. Causes of the decrease in size of coho salmon (*Oncorhynchus kisutch*). Can. Tech. Rep. Fish. Aquat. Sci. No. 971.

Ricker, W.E., Bilton, H.T., and Aro, K.V. 1978. Causes of the decrease in size of pink salmon (*Oncorhynchus gorbuscha*). Tech. Rep. Fish. Mar. Serv. Can. No. 820.

Rogers, D.E. 1980. Density-dependent growth of Bristol Bay sockeye salmon. *In* Salmonid ecosystems of the North Pacific. *Edited by* W.J. McNeil and D.C. Himsworth. Oregon State University Press, Corvallis, Oreg. pp. 267–283.

Rogers, D.E. 1994. Forecasts of the 1995 sockeye salmon runs to Bristol Bay. INPFC Doc. No. FRI-UW-9417. Available from D. Rogers, Fisheries Research Institute, University of Washington, Seattle, WA 98195.

van den Berghe, E.P., and Gross, M.R. 1989. Natural selection resulting from female breeding competition in Pacific salmon (*Oncorhynchus kisutch*). Ecology, **43**: 125–140.

Welch, D.W., and Morris, J.F.T. 1994. Evidence for density-dependent marine growth in British Columbia pink salmon populations. Document submitted to the Annual Meeting of the North Pacific Anadromous Fish Commission (NPAFC), Vladivostok, Russia, October 1994. NPAFC Doc. No. 97.Available from D. Welch, Pacific Biological Station, Nanaimo, BC V9R 5K6, Canada.

Welch, D.W., and Noakes, D.J. 1993. Trends in catch and average size of Pacific salmon in Canada, with a report on 1992 escapement levels. Document submitted to the Annual Meeting of the North Pacific Anadromous Fish Commission, Vancouver, B.C., November 1993. Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C.

ERRATUM

Erratum: Identification of larval *Contracaecum osculatum* s.l. and *Phocascaris* sp. (Nematoda: Ascaridoidea) from marine fishes by allozyme electrophoresis and discriminant function analysis of morphometric data

John Brattey

Ref.: Can. J. Fish. Aquat. Sci. 52(Suppl. 1): 116–128 (1995).

On pages 120 and 123, addition symbols were inadvertently substituted for multiplication symbols between the coefficients and squared terms of equations 1, 3, and 4, which should read as follows:

[1] $D_i^2(x) = [a + (b \cdot LN) + (c \cdot LN^2) + (d \cdot DM) + (e \cdot DM^2) + (f \cdot DC) + (g \cdot DC^2) + (h \cdot (LN \cdot DM)) + (i \cdot (LN \cdot DC)) + (j \cdot (DC \cdot DM))]$

where $D_i^2(x)$ is the generalized squared distance, LN is the nematode length, DM is the diameter at the midpoint, DC is the diameter at the base of the caecum, and *a*–*j* are coefficients for each nematode species generated from training data sets.

[3] *C. osculatum* B: $D_i^2(x) = [-38.734 + (1.317 \cdot LN) + (-0.158 \cdot LN^2) + (74.940 \cdot DM) + (-122.410 \cdot DM^2) + (50.892 \cdot DC) + (-144.264 \cdot DC^2) + (3.812 \cdot (LN \cdot DM)) + (1.232 \cdot (LN \cdot DC)) + (77.404 \cdot (DC \cdot DM))]$

[4] *Phocascaris* sp.: $D_i^2(x) = [-16.483 + (0.692 \cdot LN) + (-0.088 \cdot LN^2) + (12.501 \cdot DM) + (-147.846 \cdot DM^2) + (28.813 \cdot DC) + (-171.794 \cdot DC^2) + (4.260 \cdot LN \cdot DM)) + (0.872 \cdot (LN \cdot DC)) + (184.424 \cdot (DC \cdot DM))]$

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